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OCULAR CHANGES PRODUCED BY TOTAL BODY IRRADIATION*

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The effects of instantaneous total body irradiation on the eye had not been explored in advance of the atomic bomb explosions in Japan. Since that time a number of articles have appeared which suggest clinical and pathologic patterns of ocular damage. Flick,[1] who made a clinical study of the eyes of survivors, found retinal lesions in 46 of approximately 400 patients. He estimated that the retina was damaged in about 50 per cent of those with clinical signs of total body irradiation. Ophthalmoscopic examinations revealed 10 patients with retinal exudate alone, 14 with hemorrhage alone, and 22 with both exudate and hemorrhage. The flame hemorrhage was the type most commonly seen. Leukopenia, a feature in all who suffered total body irradiation, was more severe in patients with both hemorrhage and retinal exudate than in those with exudate or hemorrhage alone.

Schlaegel[2] reported the histopathologic features of 6 eyes and of one other lens from 7 patients who died at the Kyushu Imperial University. The lenticular changes consisted of thickening of the posterior capsule and the formation of vacuoles in the anterior and posterior subcapsular cortex. Although he admitted the possibility that these changes represented early irradiation cataracts, he called attention to Bellows'[3] statement that it is the anterior capsule which thickens following irradiation. He also suggested that anoxia may have contributed to the production of the vacuoles present in varying numbers in all 7 lenses which he examined. In one eye the vessels were distended with mononuclear cells; other changes attributed to the systemic effects of irradiation were serous exudation from the ciliary body, bacilli in the vessels of the eye, septic choroiditis,[4] and cellular infiltration of the retina.

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Wilder[5] found septic choroiditis in 17 of 18 eyes of atomic bomb casualties from Hiroshima. Conjunctival hemorrhage, preretinal hemorrhage, and choroidal hemorrhage were each seen once. Bacilli engorged the choriocapillaris of one eye. Fifteen of 16 lenses examined exhibited degenerative changes consisting of swelling of fibers at the posterior pole and large vacuoles beneath the capsule. Focal areas of swollen nerve fibers and cytoid bodies, believed to correspond to the exudates observed clinically by Flick,[1] were present in one retina.

Warren and Draeger,[6] in their study of the general bodily injuries produced at Hiroshima and Nagasaki, observed that the ionizing radiation effects of atomic fission are comparable to those of high voltage roentgen or radium radiation.

Warren[7] described two stages of radiation injury resulting from atomic explosion. The immediate stage was characterized by weakness, malaise, and fever, frequently followed by death. The delayed effect was most striking in those structures known to be especially radiosensitive: the hematopoietic and lymphoid tissues. Hemorrhagic deaths associated with thrombocytopenia were common. In this connection, Allen and Jacobson[8] demonstrated the presence in the blood of a substance with the properties of heparin, which may, at least in part, be responsible for the hemorrhagic manifestations. Anemic manifestations appeared in patients who survived longer than a few weeks. Infection often was associated with leukopenia. Liebow, Warren, and DeCoursey,[9] in an exhaustive study, illustrated and tabulated in chronologic sequence the general pathologic changes in atomic bomb casualties.

Operation Crossroads (Bikini) provided an opportunity for study of the effects of massive ionizing radiation on experimental animals. Tullis and Warren[10] stated that the animals died, as a rule, in the acute stage of radiation sickness, usually within 2 weeks. According to the observations of these authors and those of Cronkite,[11] the animals were normal in appearance and behavior at first, but after 2 or 3 days exhibited anorexia, diarrhea, and extreme irritability. The total leukocyte count rapidly diminished. Clotting and prothrombin times increased. Bloody diarrhea was common. The lesions observed at autopsy were of three types: hemorrhagic, infective, and degenerative, resulting in ulcers of the gastro-intestinal tract and tonsils.

Later, Tullis[12] compared the generalized lesions produced in experimental animals by total body irradiation during atomic bomb tests with those following total body exposure to million volt roentgen radiation.

The object of the present study has been twofold: (1) correlation of the changes in the eyes of experimental animals exposed to ionizing

rays at Bikini with those in the eyes of atomic bomb casualties from Hiroshima and Nagasaki; (2) comparison of the ocular changes caused by atomic fission radiation with those caused by total body roentgen irradiation.

Material

The material available for examination was derived from animals in three categories: (1) 21 goats and 31 pigs receiving varying amounts of total body ionizing radiations at Operation Crossroads; (2) 24 pigs receiving total body roentgen radiation, and 4 fetuses from roentgen radiated sows; (3) 24 control animals. The swine used at Bikini were 3 to 4 months of age at the time of exposure; the goats, 3 to 4 years. The controls were herd mates of the irradiated animals and included some which had been aboard target vessels, but at too great a distance from the explosion to receive any calculated radiation. The 24 pigs subjected to total body roentgen radiation were from a single herd and from 12 to 15 months old at the time of exposure. With the exception of the fetuses from which both eyes were taken, only one eye was obtained from each animal.

The Bikini Experiment

The 52 exposed animals used at Bikini received varying degrees of ionizing radiations. The pigs placed on ships for the underwater explosion test (Baker) were shielded from blast and heat, whereas some of the goats receiving heavy radiation in the air explosion test (Able) were subjected to blast. Seventeen of the controls, referred to as target controls, were aboard target ships which were calculated to be at too great a distance from the explosion to be affected by the rays. The other 7 were on the laboratory ship and so were not exposed.

All heavily and moderately irradiated animals died from radiation sickness between 3 and 23 days following exposure, with the exception of 2 heavily irradiated pigs which were killed by exsanguination, one on the 6th, and one, already moribund, on the 8th day. Three slightly irradiated goats died: one on the 4th day with atelectasis, one on the 9th day with clear symptoms of radiation sickness, and one on the 184th day with bilateral bronchopneumonia. Four were killed: one on the 7th day, one on the 9th, and 2 on the 168th day. All animals in this group, except 2 on which blood studies were not made, were known to have had a transitory reduction in total leukocyte count. The slightly irradiated pigs were shielded more completely than were the slightly irradiated goats, and at autopsy there was no gross evidence of radiation sickness in the pigs. With one exception they were killed by exsangui-

nation: one on the 46th day, the others between 200 and 214 days following exposure. One pig died of internal hemorrhage at the site of venipuncture on the 59th day.

The eyes from the animals irradiated at Bikini showed many changes similar to those in other organs [12] as well as to those found in the eyes of human casualties. The blood vessels of these animals contained very few leukocytes, mostly of the mononuclear type, with occasional large, bizarre forms. This is consistent with the depression of bone marrow activity described by Cronkite [11] and by Tullis.[12]

Hemorrhagic phenomena were observed in 5 of 36 heavily and moderately irradiated animals. The hemorrhages were distributed in the conjunctiva, choroid, retina (Fig. 1), and, in one instance, in the anterior chamber. Intra-ocular serous exudates were present in 2 animals receiving slight radiation. Serous exudate was suprachoroidal in one instance and subretinal in the other.

Septic choroiditis, indicated by infiltration of mononuclear cells, had occurred in one heavily irradiated goat (Fig. 2). Bacteria were present in the ciliary body and choroid (Fig. 3) of 8 goats: in one of 7 receiving a minimal dosage, in 3 of 8 receiving a moderate dosage, and in 4 of 6 receiving a heavy dosage. The organisms were bacilli in 6 instances, cocci in one, and both bacilli and cocci in another. In one of the 22 pigs exposed to heavy radiation, the vessels of the orbit, conjunctiva, and retina were loaded with bacilli. This was the only porcine eye in which bacteria were found.

Small vacuoles were seen in 25 of the 47 lenses which were available for examination (Fig. 4). They occurred most often at the equator in the region of the nuclear bow, but the anterior and posterior cortical fibers were not always spared. These vacuoles resembled those noted by Schlaegel [2] in the lenses of casualties from Nagasaki. Diffuse cortical degeneration (Fig. 5), with liquefaction and occasional morgagnian globules and disturbance of the subcapsular epithelium, was found in the lenses of 2 heavily irradiated animals dying of radiation sickness.

The Roentgen Radiation Experiment

The eyes of 24 swine receiving total body roentgen radiation of from 200 r. to 600 r. (Tullis [12]) were compared with eyes of the Bikini animals and the controls. Eight had hemorrhages variously distributed in the orbit, conjunctiva, retina (Fig. 6), and vitreous. Serous exudates were present in 2 hemorrhagic eyes and in 4 eyes without hemorrhage. They were subchoroidal, subretinal, and preretinal. Two pigs showed septic choroiditis. Bacteria were found in the uveal vessels of 3 ani-

mals, in one of which the central retinal veins also contained bacilli. The lenticular changes were comparable to those in animals of the Bikini group. Small cortical vacuoles, predominating at the equator, were present in 11 of the 24 animals, and in 3 there was marked diffuse posterior subcapsular degeneration (Fig. 7). The pathologic changes were present in the eyes of the lightly as well as the heavily irradiated animals.

At the time of roentgen radiation several of the pigs were pregnant. The eyes of 2 fetuses from one of these sows receiving 400 r., and of 2 fetuses from another sow receiving 600 r., were available for study. Whereas the eye of the sow which received 400 r. showed only slight preretinal edema and small vacuoles in the cortex of the lens, hemorrhages and lenticular changes were prominent in the eyes of the 2 fetuses, which were of approximately 3 months' gestation. Massive subretinal hemorrhage with complete retinal detachment had occurred in both eyes of the 2 fetuses (Figs. 8 and 9). The conjunctiva and orbit also were hemorrhagic. There was advanced degeneration of the deep fibers of the lens. In the other pregnant pig, which received 600 r., there was early posterior subcapsular degeneration of the lens, but no other change which could be interpreted as pathologic. The 2 fetuses available from this pig were slightly younger than the others. Moderate hemorrhage in the nerve head was present in one of these 4 eyes, but the massive retinal hemorrhages seen in the fetal eyes previously described were lacking. Extensive degenerative changes had taken place throughout all 4 lenses (Fig. 9). A broken lens capsule with hemorrhage from the tunica vasculosa lentis into the lens was observed in 3 of these eyes.

The Control Animals

The control animals died or were killed at varying intervals over a period of 7 months following test Able. Nine of the control and target control pigs were killed by exsanguination, and one by sodium pentothal. None showed significant gross changes at autopsy. Three died from pneumonia and 2 from undetermined causes. Seven control and target control goats were killed by exsanguination, and at autopsy no significant gross changes were observed. Two died: one with pulmonary congestion but no other gross evidence of disease; the other, a target control animal, had edema of the neck organs and petechiae in the head and large intestine. Scleral hemorrhages were described for this animal at autopsy but were not identified microscopically. Examination of this animal's eye revealed septic choroiditis and a small perivascular retinal hemorrhage. Many polymorphonuclear leukocytes in a number

of intra-ocular vessels indicated an inflammatory process unrelated to aplastic anemia. Eyes of other control animals were without these manifestations, and bacteria were not found in any of them. Small vacuoles in the cortex (Fig. 10) appeared in 13, or 65.0 per cent, of the 20 lenses of control animals available for study, as compared to 57.5 per cent of the 47 lenses from animals radiated at Bikini, and 58.3 per cent of the 24 lenses from roentgen radiated animals. It was impossible to correlate their presence with age, manner of death, or extent of post-mortem degeneration. Since vacuoles were present in both irradiated and control animals, they cannot be attributed to radiation effects alone. Advanced diffuse cortical degeneration (Fig. 11) was present in 2 control pigs: one a poorly nourished runt dying of pneumonia, the other a target control pig killed 9 days after the explosion and showing no significant pathologic changes at autopsy.

Discussion

Certain of the lesions in eyes of animals receiving total body irradiation, whether from atomic bomb explosion, or roentgen rays, were similar to those described in the eyes of the atomic bomb casualties from Hiroshima and Nagasaki.[1,2,5] These included conjunctival and intra-ocular hemorrhages, intra-ocular serous exudates, septic choroiditis, and bacteria in the intra-ocular vessels, all of which were attributed to bone marrow changes with resultant leukopenia and to subsequent severe infections. The bacteria were limited to the lumina of vessels, and while they were not regarded as post-mortem contaminants, it is possible that they increased greatly in number after the death of the host.

Five lenses from heavily irradiated animals, 2 from the Bikini group and 3 which received roentgen radiation, as well as the lenses of fetuses from heavily irradiated sows, showed extensive degeneration. In the older animals the changes were confined to the cortex, but in the fetal eyes the nucleus was affected also. Cortical degeneration was present in the lenses of 2 control animals, one of which was an undernourished pig dying of pneumonia and the other an apparently normal pig. Most of the changes in the lens were limited to the fine cortical vacuolation described by Schlaegel [2] in lenses of casualties from Hiroshima and Nagasaki. Their presence in control animals indicates a cause other than irradiation, possibly anoxia or even post-mortem change. They closely resembled the reversible changes in the lens following asphyxia, which Biozzi [13] described in experimental rats. The large posterior subcapsular vacuoles described by Wilder[5] were not seen. Thickening of the posterior lens capsule, described by Schlaegel in human atomic bomb casualties

and in experimental animals following roentgen and radium radiation, was not identified. It is interesting to note at this point that evaluation of the thickening of the anterior capsule in swine would be difficult, as it is a normal phenomenon of growth. The observations in regard to cataract following irradiation are by no means final. All of the animals used in this study died or were killed within 6 months of exposure; thus it may be that prolonged investigations of animals receiving sublethal irradiation would reveal delayed development of cataracts such as occurs in human beings who have received local irradiation to the head. The massive cataractous process in the irradiated fetus was in marked contrast to the minimal evidence of cataract in most of the adult animals. The exposure occurred when the tunica vasculosa lentis was still present. The lens at this time is in a period of most rapid growth and metabolic activity, and would, therefore, be most susceptible to deficiency in blood supply and nourishment. The rapid development of cataracts in fetal eyes following total body irradiation of the mother is in agreement with the finding of cataracts in newborn animals following local roentgen radiation of the eyes.[3,14-16] In like manner, irradiation of a pregnant animal would be expected to affect other organs of the fetus which were undergoing most rapid growth and differentiation at the time of exposure. A higher rate of abortions and a greater proportion of abnormalities in the live-born would be anticipated.

Superficial corneal lesions attributed by Schlaegel [2] to direct irradiation and by Wilder [5] to direct injury were not seen. As pigs and goats do not have a Bowman's membrane, no comparison could be made with the breaks in this structure in human casualties. Minimal edema and occasional desquamation of the corneal epithelium in irradiated as well as in control animals were regarded as post-mortem changes. Loss of polarity was not evident. Other changes reported in eyes from atomic bomb casualties but not observed in this series are: focal swelling of retinal nerve fibers with cytoid bodies,[1,5] perivascular infiltration of the retina by mononuclear cells, distention of vessels by leukocytes, and fibrin nests on the surface of the retina.[2]

Post-mortem degeneration was advanced in some eyes. It was, however, not regarded as responsible for any of the changes described in this paper except edema and desquamation of the corneal epithelium. It may possibly have contributed to the fine vacuolation of cortical fibers of the lens.

Conclusions

Pathologic changes in the eyes of experimental animals receiving total body irradiation from atomic fission were similar to those resulting from

roentgen radiation. For the most part, they were regarded as results of bone marrow depression and secondary systemic changes, rather than as direct effects of the rays. The hemorrhages were attributed to thrombocytopenia and to the heparin-like substance demonstrated by Allen and Jacobson.[8] Serous exudates were the result of increased vascular permeability, possibly secondary to anemia. Septic choroiditis and bacteria in the vessels were manifestations of the septicemia. Vacuoles similar to those observed by Schlaegel[2] were present in the lenses of many of the irradiated animals but were found also in the controls and therefore were not regarded as a specific manifestation of irradiation. Massive cataract in irradiated animals may have been due to direct radiation, although secondary nutritional effects associated with the anemia and severe hemorrhages could not be ruled out. Immediate changes in the lens specific for irradiation were not apparent in the young or mature animal. Knowledge of latent changes must await further experimentation. Although the secondary effects of ionizing and roentgen radiation were similar in the eyes of both pigs and goats, there were no ocular changes which could be attributed unequivocally to direct irradiation.

We wish to express appreciation to the staff of the Naval Medical Research Institute and Operations Crossroads for making the material available for study.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Perivascular hemorrhage in the retina of a goat dying with radiation sickness 16 days after exposure to ionizing radiation at Bikini. Armed Forces Institute of Pathology accession no. 248001 (G 150). × 114.

FIG. 2. Mononuclear cells in the vascular layer of the choroid of the same goat as used for Figure 1. A.F.I.P. acc. 248001 (G 150). × 114.

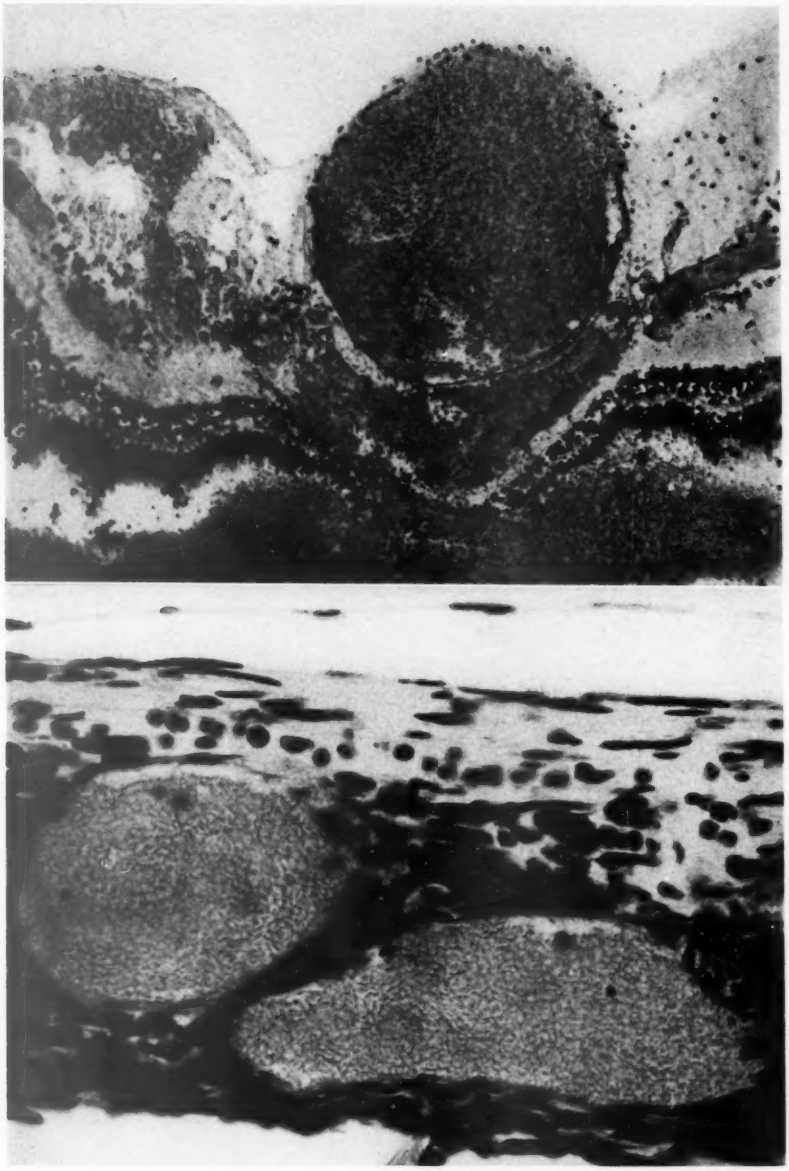
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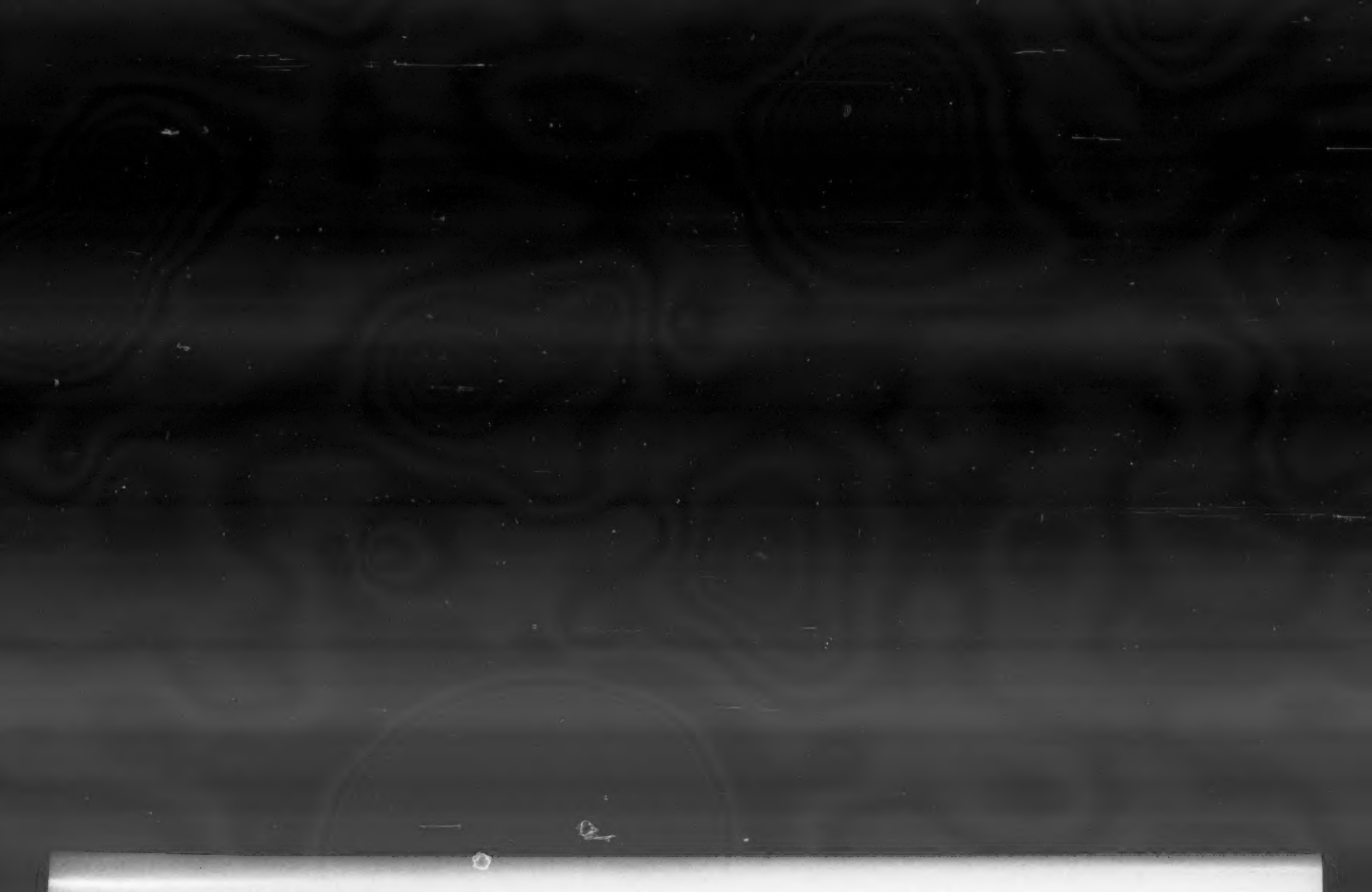
PLATE 2

FIG. 3. Bacilli in a large choroidal vein of a goat dying with radiation sickness 5 days after exposure to ionizing radiation at Bikini. A.F.I.P. acc. 248001 (G 12). × 330.

FIG. 4. Small vacuoles in the region of the nuclear bow in the lens of a goat dying with radiation sickness 9 days after exposure to ionizing radiation at Bikini. A.F.I.P. acc. 248001 (G 60). × 280.



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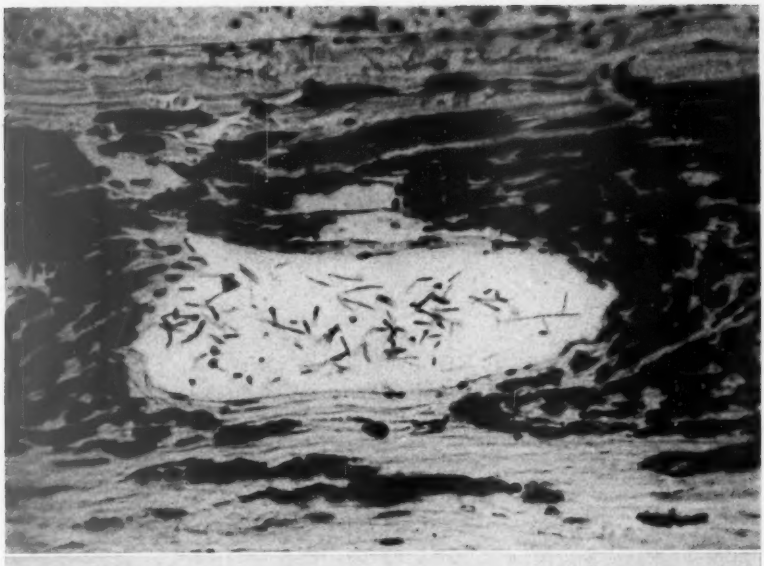

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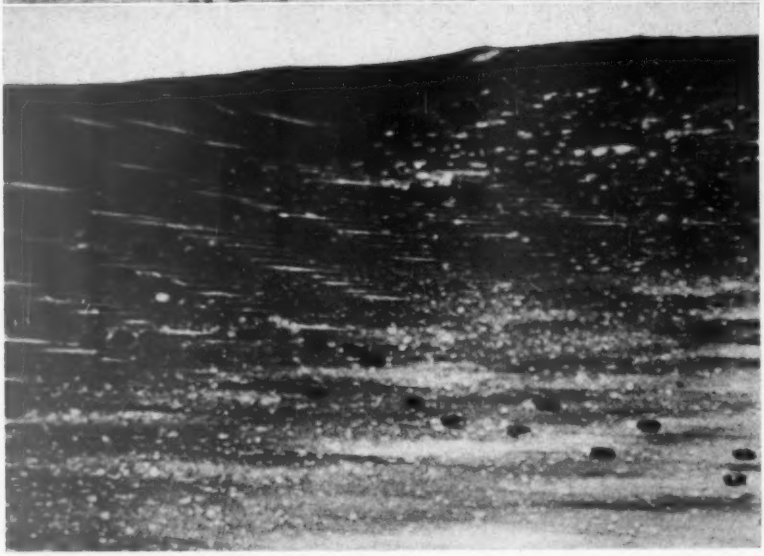


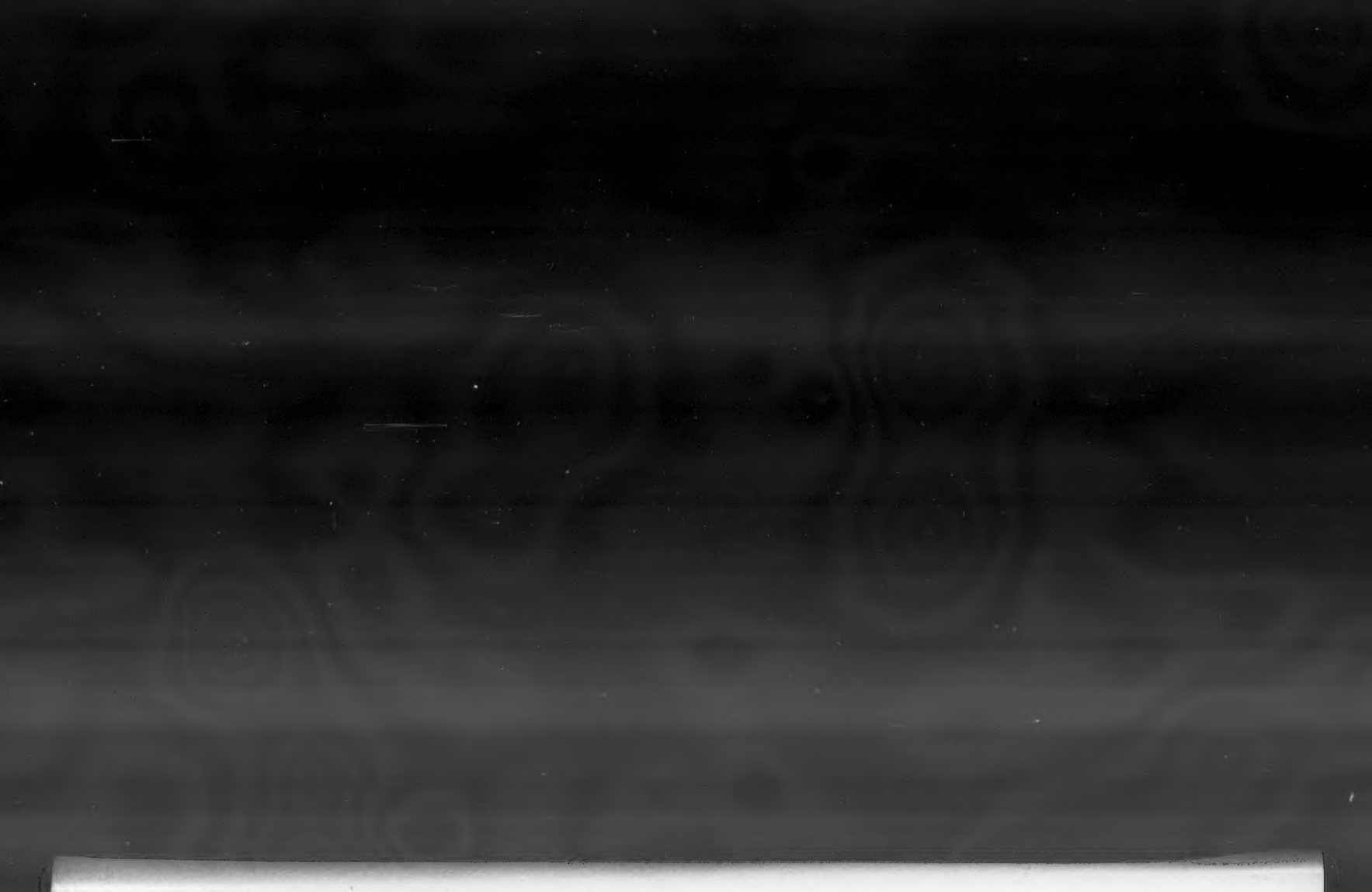
Wilder and Maynard Ocular Changes Produced by Irradiation

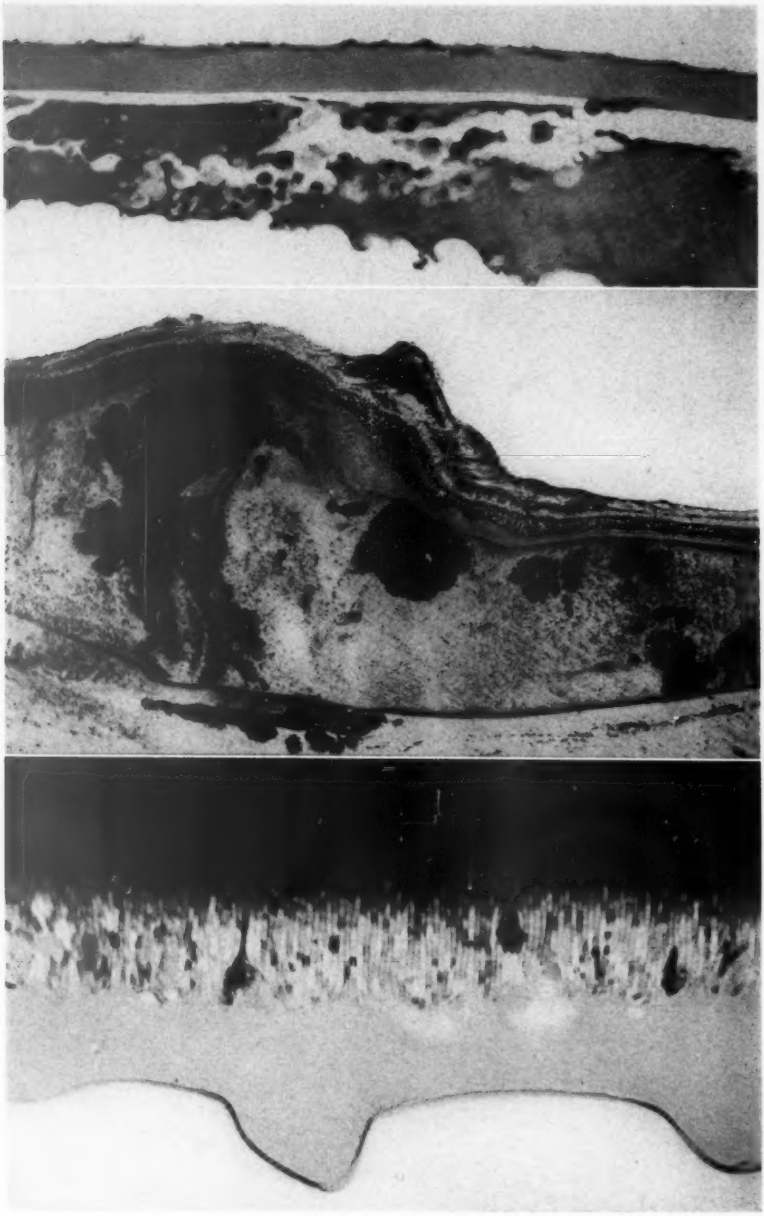
PLATE 3

FIG. 5. Anterior cortical degeneration with disorganization of the subcapsular epithelium in the lens of a pig dying with radiation sickness 11 days after exposure to ionizing radiation at Bikini. A.F.I.P. acc. 248001 (P 420). × 180.

FIG. 6. Retinal hemorrhages in the eye of a pig dying with radiation sickness 26 days after exposure to roentgen radiation (400 r.). A.F.I.P. acc. 248002 (XP 490). × 24.

FIG. 7. Diffuse degeneration of the posterior subcapsular lens fibers in a pig dying with radiation sickness 14 days after exposure to roentgen radiation (400 r.). A.F.I.P. acc. 248002 (XPA). × 180.





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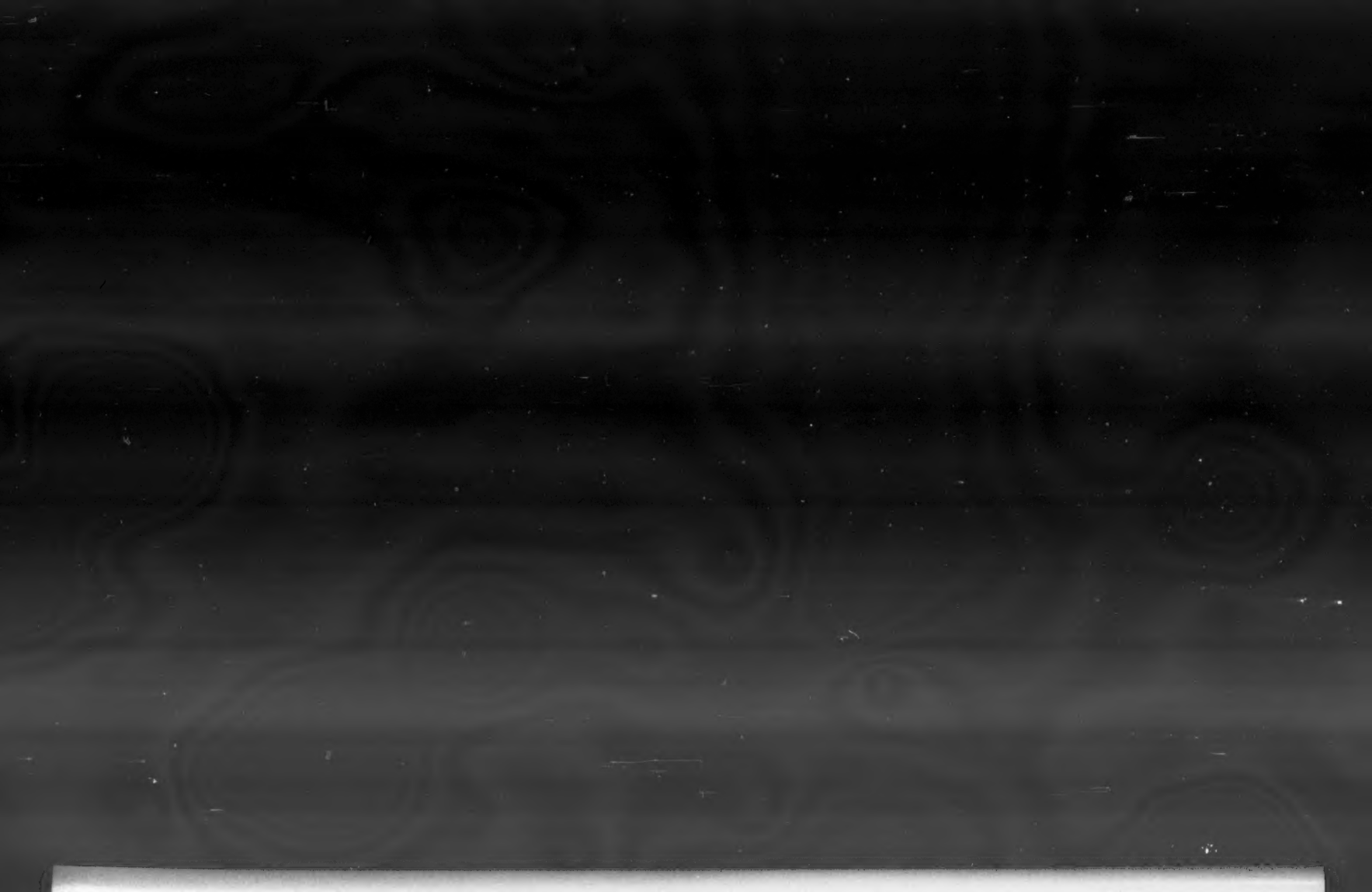
Wilder and Maynard Ocular Changes Produced by Irradiation

PLATE 4

FIG. 8. Subretinal hemorrhage and degeneration of the nuclear and deep cortical lens fibers in the eye of a fetal pig whose mother received total body roentgen radiation (400 r.). A.F.I.P. acc. 248002 (XP 461). × 10.

FIG. 9. Degeneration of the entire lens of a fetal pig whose mother received total body roentgen radiation (600 r.). A.F.I.P. acc. 248002 (XPC). × 40.



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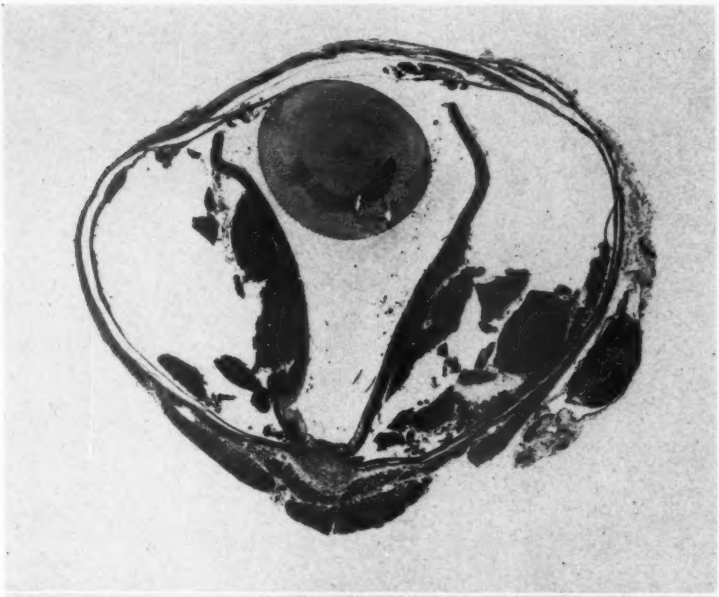

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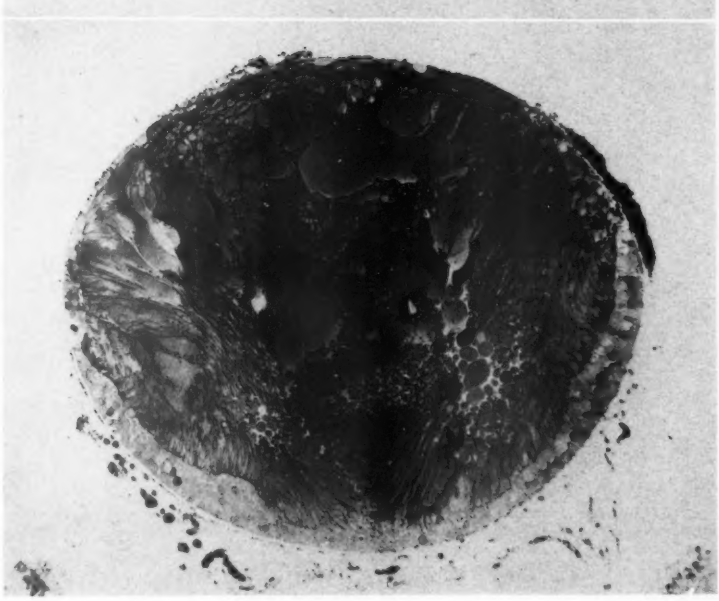

Wilder and Maynard Ocular Changes Produced by Irradiation

PLATE 5

FIG. 10. Small vacuoles in the region of the nuclear bow in the lens of a non-irradiated control pig killed by exsanguination. A.F.I.P. acc. 248001 (P 396). × 400.

FIG. 11. Diffuse cortical degeneration in the lens of a non-irradiated target control pig killed by exsanguination and exhibiting no gross lesions at autopsy. A.F.I.P. acc. 248001 (P 221). × 75.

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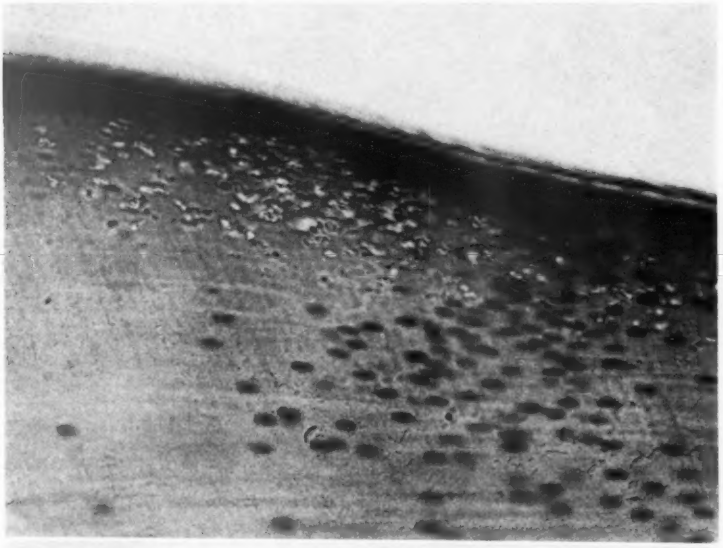

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Wilder and Maynard Ocular Changes Produced by Irradiation

THE EFFECT OF RADIOACTIVE PHOSPHORUS UPON THE DEVELOPMENT OF THE EMBRYONIC TOOTH BUD AND SUPPORTING STRUCTURES *

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It has been shown experimentally that radioactive phosphorus‡ administered to a pregnant mouse results in the uptake of this material by the offspring.[1] It is recognized also that rapidly growing normal [1] as well as neoplastic [2] tissues show a high and rapid uptake of P^{32}.

Previous work has shown that the internal administration of radiophosphorus to young mice results in marked alterations of structure and function of the odontogenic apparatus.[3] On this basis it was decided to ascertain the effect of internal beta radiation on the developing tooth structure of the embryo. The small amounts of radiophosphorus metabolized in the embryonic bone and tooth structure subject these developing tissues to continuous high speed electron bombardment.

The mouse has first, second, and third molar teeth, the post-natal development of which is limited to the first month of life. The histogenesis and structure of these teeth bear a close similarity to those of human dentition. Development of the first and second molar teeth begins during the later stages of gestation while that of the third molar teeth commences about 5 days following birth. The incisor teeth grow continuously from persistent pulps as is the case with all rodents.

Methods

Eighteen pregnant C57 black mice were given one injection subcutaneously of a carrier-free solution of radioactive phosphorus (P^{32} combined as phosphoric acid) with dosages ranging from 5 to 17 μc./gm. of body weight (0.25 to 1.0 cc. of solution). These animals were so treated 1 to 5 days before parturition. Following birth of the litters the immature mice were sacrificed at intervals of 6, 12, 14, and 28 days. Those animals which appeared moribund were sacrificed at earlier intervals. Offspring of pregnant mice injected with non-radioactive phosphoric acid (approximately 0.06 N.) served as controls. The heads were fixed in 4 per cent formaldehyde, decalcified in 10 per cent formic acid, and embedded in paraffin. The teeth and supporting structures were

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‡ The P^{32} used in this experiment was supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, U.S. Atomic Energy Commission.

sectioned in a sagittal plane while the mandibular joint was sectioned in a frontal plane. All tissues were stained with hematoxylin and eosin.

Results

Litters were born from 2 to 6 days following injection of the mother. All litters (except offspring from mice receiving 5 μc./gm. of body weight) appeared undersized and those which were born at longer intervals following treatment of the mother remained conspicuously dwarfed. With dosages of 10 to 17 μc./gm. of body weight (pregnant mouse) the offspring at 10 days of age appeared no larger than at birth. With 5 μc./gm. of body weight the litters appeared normal at birth and continued to develop in a normal fashion.

Microscopic Observations

15 to 17 μc./gm. of Body Weight of Pregnant Mouse

Litters were born 1 and 4 days following administration of 15 to 17 μc./gm. of body weight. Microscopic study of the latter group 6 days after birth revealed the following changes. The first and second molar teeth were rudimentary and had a prominent stellate reticulum. The ameloblasts adjacent to the occlusal area of the first molar appeared relatively normal. However, toward the root portion of the crown they appeared as squamous epithelium. No enamel or enamel matrix was visible. The odontoblastic layer was not intact and many of the odontoblasts appeared as spindle-shaped cells. There was a small amount of dentinoid matrix between the ameloblasts and the pulp. This matrix was homogeneous (Figs. 1 and 2). No evidence of dentin or enamel formation was visible in the second molar. The cells of the inner enamel epithelium (ameloblastic layer) appeared as low-cuboidal or squamous epithelium. The pulp was aplastic and contained a few macrophages (Figs. 3 and 4).

The incisor teeth exhibited normal enamel formation. The odontoblastic layer, however, was distorted and there was osteodentin formation in the pulp chamber (Figs. 5 and 6). The blood vessels of the pulp were dilated and the stroma exhibited hydropic change.

There was a marked marrow aplasia of the alveolar bone (Fig. 5). Osteoblastic activity was markedly diminished and the marrow exhibited early fibrosis with scattering of multinucleated giant cells and macrophages. The new fibrous bone was more basophilic in staining than is normal and exhibited prominent cement lines.

The cranial portion of the mandibular joint appeared relatively normal except for aplasia of the marrow. The interarticular disk likewise was normal. The condyle was small and underdeveloped as compared

with normal controls, and the number of osteoblasts was reduced. The hypertrophic and proliferating cartilage cells were enlarged abnormally. The nuclei of some of the chondrocytes were not visible, while the nuclei of others were shrunken and stained darkly. There was aplasia of marrow along with spindle cells and extravasated erythrocytes. Numerous osteoclasts were present in the acellular marrow space adjacent to the zone of erosion. Trabecular formation was lacking and the bony cortex and periosteum were thin (Figs. 7 and 8).

Those animals which were born 1 day following maternal injection and which were therefore in a later developmental stage when subjected to the action of P^{32}, exhibited changes similar to the previous series with the following exceptions. At 6 days of age the structure of the first molar tooth showed a continuous ameloblastic layer, enamel matrix, and a continuous layer of normal dentin. Toward the root portion of the crown the ameloblasts appeared as low-cuboidal cells and the continuity of the odontoblastic layer was lost. Instead, there was an irregular pattern of osteodentin within which were trapped abnormal spindle-shaped odontoblasts.

The second molar tooth, however, was similar to that described in the previous series. The stellate reticulum was still prominent and the ameloblasts appeared as cuboidal cells. A trace of dentin matrix was visible. However, no normal odontoblasts were present. The stroma of the pulp was aplastic and some of the cells were pyknotic and exhibited hydropic changes. There was osteodentin formation at the growing end of the incisor tooth. Aplasia of marrow was similar to that described for the previous series and osteoblastic activity was depressed. However, there was considerably less immature fibrous bone than in the previous series. The changes in the mandibular joint were similar to those which have been described for other stages.

10 μc./gm. of Body Weight of Pregnant Mouse

Litters were born to mice 5 and 2 days following administration of P^{32} equivalent to 10 μc./gm. of body weight. The former group, at 12 days of age, had the ameloblastic layer of the first and second molar teeth intact except for a small area toward the apical portion of the crown. Moreover, the second molar exhibited a slight trace of enamel matrix. There was osteodentin formation in the pulp chamber of the first molar and the dentin-predentin junction was irregular in some areas. The pulp chamber of the second molar was filled almost completely by a light staining, pink, amorphous ground substance which was suggestive of early calcification. Aplasia of marrow was as severe as described in the previous series. However, the reduction in the num-

ber of osteoblasts was not as marked. The mandibular joint still had the general appearance of that of a much younger animal. The embryonic cells appeared normal while the cells of the proliferative and hypertrophic zones were considerably enlarged and the nuclei were shrunken and stained darkly. The marrow had been replaced extensively by spindle cells, and the new trabecular formation consisted of irregular and distorted fibrous bone.

In 12-day-old mice which were born 2 days following administration of radiophosphorus there were altered ameloblastic layers of both first and second molar teeth. The cytoplasm of the ameloblasts along the coronal portion of the tooth exhibited hydropic degeneration. Those ameloblasts toward the root portion of the crown appeared as low-cuboidal or squamous cells. The pulp was markedly aplastic and was filled in to a great extent by masses of osteodentin interspersed with spindle-shaped cells (Figs. 9 and 10). The histologic picture of the alveolar bone and marrow was similar to that of the preceding series. The mandibular joint likewise was similar to those of the earlier stages following the same dosage of radiophosphorus, except that there was abnormal subcartilage linkage by extensive immature bone formation (Figs. 11 and 12).

5 μc./gm. of Body Weight

Maternal administration of 5 μc./gm. of body weight produced no alteration in the development of tooth structure. However, there was a moderate aplasia of the marrow of both alveolar and mandibular bone, but the number of osteoblasts appeared normal. At 1 month of age the histologic picture appeared normal.

0.06 N. Non-Radioactive Phosphoric Acid

The teeth and jaws of control animals from mothers injected with 0.25 to 1 cc. of 0.06 N. non-radioactive phosphoric acid exhibited no histologic abnormalities.

Discussion

Dosages of 10 μc./gm. of body weight of P^{32} administered to young mice are not sufficient to produce the alterations described. The fact that 10 μc./gm. of body weight administered to a pregnant mouse can produce such marked changes in the osteogenesis and odontogenesis in the offspring can be explained in two ways: (1) that the embryonic tooth and supporting structures exhibit a greater radiosensitivity; and (2) that because of the rapid growth of embryonic tissue, the uptake of P^{32} is such as to concentrate this radioactive material in tissues, thus producing a greater intensity of radiation. The most marked altera-

tions of odontogenic structure and function are produced by administration of 15 to 17 μc./gm. of body weight to the pregnant mouse.

The degree of damage is also dependent upon the stage of development of the animal *in utero,* or, in other words, the stage of histogenesis and morphogenesis of the dentition. In all of these animals, histodifferentiation of the first and second molars has already commenced before administration of radiophosphorus. Uptake of this radioactive material in the developing tooth primordium resulted in a failure of further differentiation and also alteration in cellular structure.

The lack of development of the second molar teeth (Fig. 3) suggests that the inner enamel epithelium or perhaps the mesenchymal pulp has been damaged so that differentiation of odontoblasts and initiation of dentin formation is inhibited. The changes in the condyle indicate an abnormal degeneration of proliferating and hypertrophic chondrocytes with a concomitant inhibition of normal osteogenesis and ossification. The general outline of the condyle is immature in appearance yet the early subcartilage linkage and degeneration of the chondrocytes suggest premature ageing.

It is observed that the greatest odontoblastic and ameloblastic distortion is present toward the root portion of the crown where these cells have differentiated most recently. This suggests that newly differentiated cells are much more radiosensitive than older cells. Thus, radiosensitivity and radioresistance of the odontoblasts and ameloblasts follow a similar pattern. However, the odontoblasts are far more radiosensitive than ameloblasts.

Summary and Conclusions

Administration of P^{32} to pregnant mice results in disturbances of osteogenesis and odontogenesis in the offspring. The dosages required to produce these changes are less than are required to produce similar changes by injecting immature mice.

With dosages of 10 to 17 μc./gm. of body weight of mother, there is cessation of histodifferentiation of ameloblasts and odontoblasts.

In some regions the ameloblasts appear as squamous epithelium, and are without function. The odontoblasts appear as spindle cells with cytoplasmic processes. These cells are associated with osteodentin formation.

The odontoblast is more radiosensitive than the ameloblast.

Development of the third molar tooth is completely inhibited.

Alterations in the condyle are indicative of an abnormal premature ageing process. Aplasia of marrow, premature subcartilage linkage, and decreased osteoblastic activity are characteristic changes.

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DESCRIPTION OF PLATES

All sections were stained with hematoxylin and eosin, and photographed at a uniform magnification of × 75.

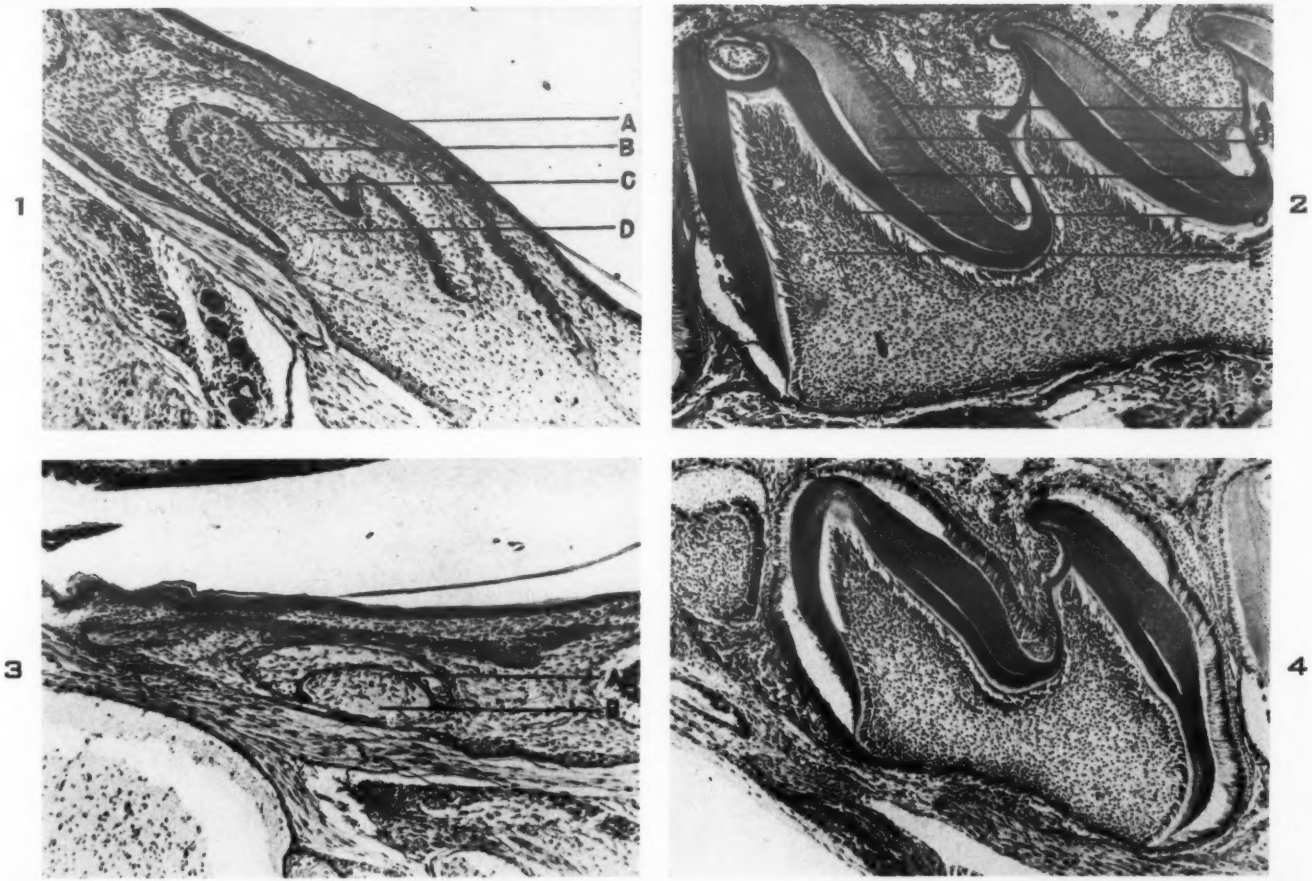
PLATE 6

FIG. 1. First molar tooth of a 6-day-old mouse, the mother of which received P^{32}. A=ameloblasts, B=thin layer of dentinoid matrix, C=disorganized odontoblastic layer, D=pulp.

FIG. 2. First molar tooth of a normal 6-day-old mouse, showing well developed enamel and dentin. A=ameloblastic layer, B=enamel, C=dentin, D=odontoblasts, E=pulp.

FIG. 3. Second molar tooth of a 6-day-old mouse, the mother of which received P^{32}. No enamel or dentin is present, and the ameloblasts (A) are low-cuboidal cells. B=pulp.

FIG. 4. Second molar tooth of a normal 6-day-old mouse.



Burstone

Effect of P^{32} on Embryonic Tooth Buds

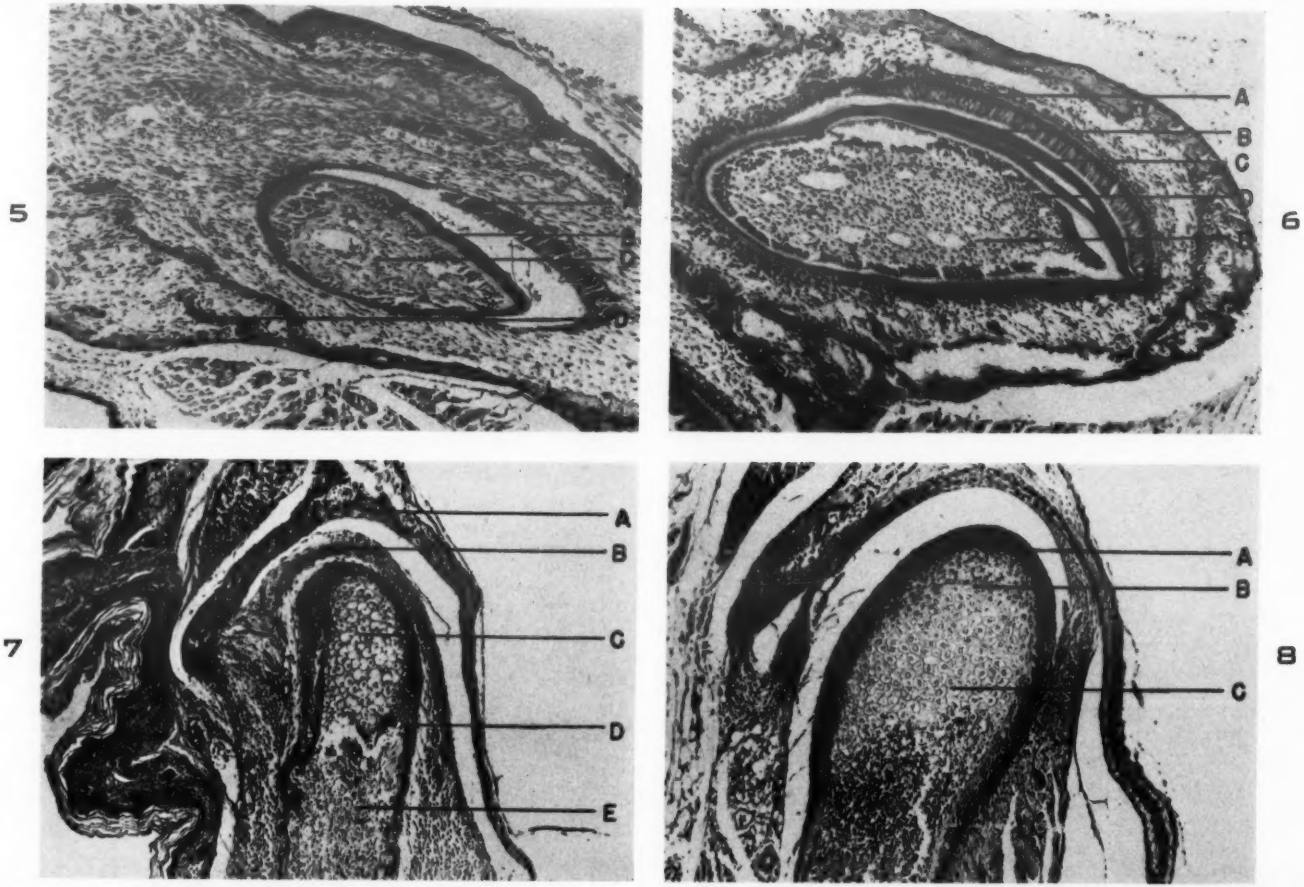
PLATE 7

FIG. 5. Cross section of an incisor tooth of a 6-day-old experimental animal. A=ameloblasts, B=odontoblasts, C=osteodentin in pulp chamber, D=fibrous marrow.

FIG. 6. Cross section of an incisor tooth of a normal 6-day-old mouse. A=ameloblasts, B=enamel matrix, C=dentin, D=odontoblasts, E=pulp.

FIG. 7. Mandibular joint of an experimental animal 6 days of age. A=cranial portion of joint, B=interarticular disk, C=enlarged cartilage cells, D=osteoclast, E=aplastic marrow and poor trabecular formation.

FIG. 8. Mandibular joint of a normal 6-day-old mouse, showing well formed trabeculae and marrow cellularity. A=embryonic zone, B=proliferative zone, C=hypertrophic zone.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A
B
C
D
6
A
B
C
D
7
A
B
C
D
E
8
A
B
C

PLATE 8

FIG. 9. Second molar tooth of a 12-day-old experimental animal. Of note is the extensive osteodentin formation in the pulp (A). B=aplastic marrow and poor bone formation.

FIG. 10. Second molar tooth of a normal 12-day-old mouse. A=enamel space, B=dentin, C=odontoblasts, D=pulp.

FIG. 11. Mandibular joint of a 12-day-old experimental animal. Of note are the reduced dimensions of the condyle and the extensive immature fibrous bone formation (A). B=osteoclast.

FIG. 12. Mandibular joint of a normal 12-day-old mouse.

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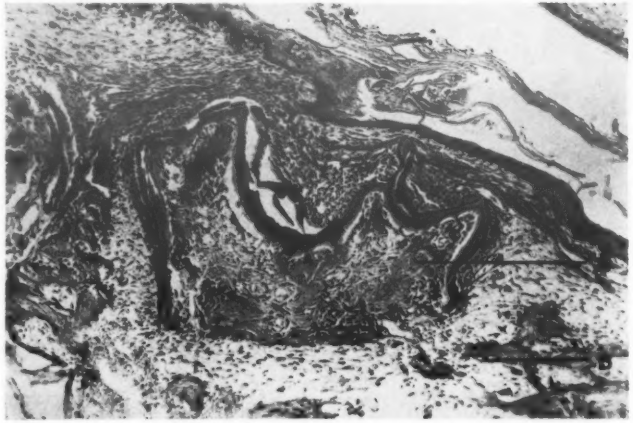

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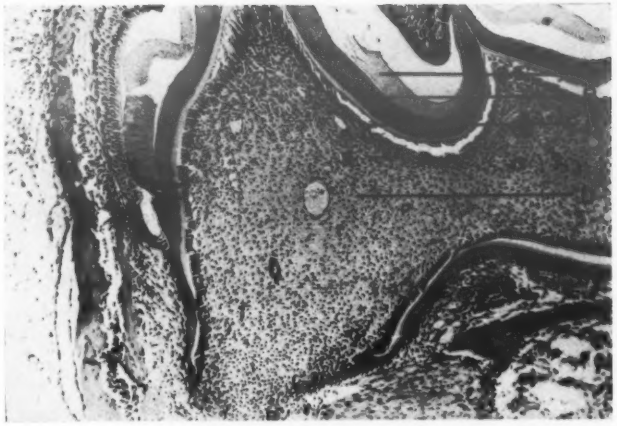

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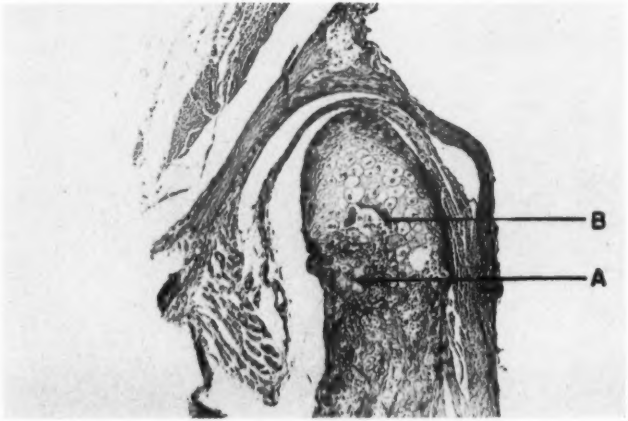

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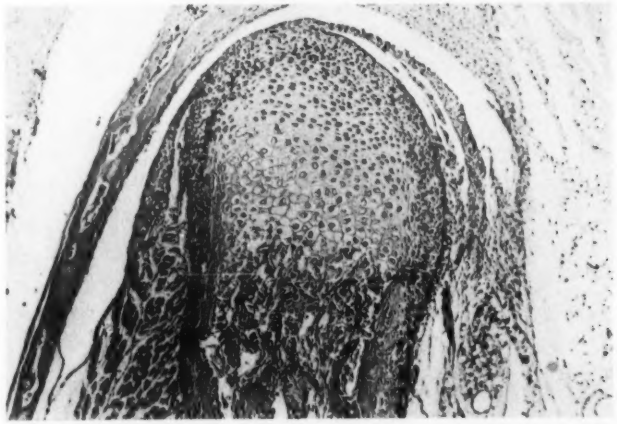

Burstone

Effect of P^{32} on Embryonic Tooth Buds

DIASTASIS AND DIASTATIC PERFORATION OF THE GASTRO-INTESTINAL TRACT

A Clinical, Pathologic, and Experimental Study *

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It is my purpose to describe, or rather to revive, a clinicopathologic entity which, though apparently well known in the latter part of the last century, has been somewhat neglected in recent years. I am referring to the train of pathologic events in one segment of the intestine which leads to distention and eventual perforation of another, proximally situated, portion of *normal* intestine, which processes may be called diastasis, and diastatic perforation.

It is well known that any part of the alimentary tract which is the seat of an ulcerative, inflammatory, traumatic, or neoplastic process may rupture at the site of the lesion. Such may be the case in ulcerative colitis, typhoid fever, volvulus, diverticulosis, foreign body impaction, or carcinoma. It is, however, not generally known that a more or less normal intestine also may rupture from overdistention alone, which is caused by an intrinsic or extrinsic obstructive lesion situated in another part of the same intestine, at a varying distance from the site of obstruction. This type of perforation, to revive the term used by Heschl in 1880, is called diastatic perforation.

By diastatic tear or perforation is meant a simple, spontaneous rupture of a non-diseased hollow viscus, occurring in the course of its progressive, sustained, and unrelieved stretching and distention, which is caused by an obstructive lesion situated in the same viscus, at some distance below the site of perforation. It does not apply to traumatic or chemical perforation nor to that which is a sequel of inflammation or tumefaction, but only to *spontaneous* rupture in a more or less normal and only secondarily distended viscus.

It is the general impression that perforation cannot occur in a normal intestine and that, therefore, whenever this occurs there must be some pre-existing pathologic lesion in the affected viscus. It will be the main thesis of this paper to show, among other things, that this impression or view is incorrect, and that a more or less normal viscus can and does rupture under the diastatic conditions as outlined above.

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Historic Considerations and Review of Literature

The first cases of intestinal distention and perforation of diastatic type were observed and reported more than 100 years ago. Corbin (1831) apparently was the first to report such a case. His graphic description of the perforated intestine corresponds in every way to our clinical and experimental findings today. Similar cases were reported by Gogué (1844), Pretty (1851), Markham (1857), Hayden (1869), Anger (1872), Goodhart (1879), and others. Heschl, in 1880, however, was the first to give this problem its due prominence. He described 2 such cases in great detail and offered an explanation for the pathogenesis of the rupture which is in agreement with our present-day concept of this syndrome. His term "diastasis" and his views on this problem have been adopted, with some modifications, by me. After publication of Heschl's work there appeared many similar case reports, although not all of the authors used the same term and only a few of them were in agreement with Heschl's view. Among the other papers dealing with this subject may be mentioned those of Wharton (1890), Luys (1899), Létulle (1899), Gandy and Bufnoir (1899), Anschütz (1902), Sauer (1902), Kreuter (1919), Guillaume (1922), van Beuren (1923), Bertrand (1924), Riche and Guibal (1929), Wakeley (1934), Saeltzer and Rhodes (1935), and Sergent, Soyer, and Leuret (1936). Some recent authors do recognize the possibility of perforation of the cecum in obstruction of the distal colon but becloud the issue by calling it "stercoral ulceration," "pericecal inflammation," etc. Most of the recorded cases and reviews of this subject have appeared in the European literature. In Germany and France this syndrome is well recognized. On the other hand, only relatively few American authors refer to diastatic rupture of the colon. "It is only within the past decade that surgeons have become alive to its importance," stated Wangensteen (1942) in his discussion on diastatic perforation of the intestines.*

Incidence

When a comparison is made between the number of cases of diastatic rupture of the alimentary tract which have been reported from 1830 to 1900 with that from 1900 to 1940, there might be gained the wrong impression that this condition is on the wane. The reason for paucity of reported cases in the recent literature is either that this condition is not recognized at all, or that such cases are usually looked upon as resulting from delay or sometimes even neglect of treatment, hence are usually not reported. Thus Sergent and associates could collect no more

* Only the more comprehensive and representative papers on this subject are mentioned here, as no useful purpose would be served by enumerating all the cases which have been reported.

than 46 cases of diastatic perforation of the cecum in the literature up to 1936, although, as they stated correctly, this condition has been encountered by many more surgeons in their career. These facts make the statistical study of the incidence of diastatic perforation of the intestines of rather doubtful value.

Report of Cases

Case 1: Diastatic Rupture of Cecum Secondary to Obstruction by a Carcinoma of the Transverse Colon

M. T. (A-104-37), a male, 58 years of age, was admitted with a 6 weeks' history of chronic constipation and recurrent attacks of abdominal pain and vomiting. He appeared cachectic. A large fluctuating mass was palpable in the right flank. Temperature was 102° F.; pulse, 100; blood pressure, 122/64 mm. of Hg; hemoglobin, 90 per cent; leukocytes, 9,000; neutrophils, 70 per cent. Obstipation persisted and all enemas were feces-free. On the fourth day he was seized with a chill, his skin became clammy, and he perspired profusely. Two hours later he expired. Clinical diagnosis: Retrocecal appendicitis with abscess formation.

Necropsy Findings. On opening the abdomen at necropsy there was an escape of gas and feces which made it appear at first as though the intestines were inadvertently punctured. The peritoneal cavity was filled with a foul-smelling, turbid fluid and many large fecal scybala. The cecum, ascending, and proximal third of the transverse colon were markedly distended (Fig. 1). The anterior surface of the cecum and ascending colon showed an elliptical perforation, 7 by 2.5 cm., which corresponded to the site and course of the torn taenia. The cecum was tissue-paper-like in thinness. Situated within the midportion of the transverse colon was a large annular tumor mass which had occluded the entire lumen. Bauhin's valve was closed. The colon distal to the tumor was empty and collapsed. Microscopically, the transverse colon showed an infiltrating adenocarcinoma, grade 3. The perforated cecum showed atrophy of all layers, but no evidence of any pre-existing inflammation (Fig. 2). The partly torn cecal wall showed areas of intramuscular and subserous organization (Fig. 2, A).

Anatomical Diagnosis. Annular, stenosing adenocarcinoma of the transverse colon with secondary distention of the cecum, ascending and transverse colon, and rupture of the cecum; diffuse fecopurulent peritonitis of the "blow-out" type; healed tuberculosis of the lungs; ureteritis cystica.

Case 2: Diastatic Rupture of the Cecum as Sequela of Intestinal Obstruction in the Course of Peritonitis Caused by a Biliary Fistula

L. Z. (A-38-38) was a male, 70 years old, who was admitted with a history of dyspnea, attacks of lumbar pains for 2 years, and severe right lumbar pain for 2

weeks. On examination, there was marked tenderness at McBurney's point. Temperature was 104° F.; pulse, 80; respirations, 28; leukocytes, 17,000; neutrophils, 60 per cent. On the third day there was marked liver tenderness. The diagnosis of retrocecal appendicitis was made. The patient refused treatment and left the hospital against advice. He was readmitted 6 weeks later in extremis. Seven hours before admission he had been seized with pain in the right chest and shoulder and vomited twice. He appeared acutely ill, dyspneic, and cyanotic. The abdomen was distended, spastic, and tender in the upper right quadrant. Temperature was 101° F.; pulse, 108; respirations, 32. A diagnosis of "mesenteric thrombosis" was made. Chemical examination of the blood showed glucose of 224 mg.; urea nitrogen, 69.6 mg.; creatinine, 4 mg. per cent. Conservative treatment was instituted. On the eighth day he became apathetic and semi-comatose. The leukocytes rose to 21,000 and neutrophils to 98 per cent. On the twelfth day he expired.

Necropsy Findings. A large amount of fecopurulent material filled the abdominal cavity. The entire intestine was adherent to the abdominal wall by plastic fibrinous exudate. The surface of the liver was covered with a thick layer of fibrin. An abscess, 4 by 1 cm., was found in the right lobe of the liver near the anterior margin. It communicated with the gallbladder through a ragged defect in its wall in which a calculus, 3 cm. in diameter, was impacted. A large rent was found in the cecum which was covered with a patch of fibrin. Microscopically, there was evidence of chronic cholecystitis and abscess of the liver. The cecum, at the site of perforation, showed edema and denudation of the mucosa but no intrinsic inflammation.

Anatomical Diagnosis. Chronic cholecystitis with rupture of the gallbladder, cholecysto-hepatic fistula, and liver abscess; intestinal obstruction, secondary to adhesion; diastatic perforation of the cecum; fecal peritonitis; diverticulosis of the ileum.

Case 3: Diastatic Tears of Taeniae of the Cecum with Carcinoma of Splenic Flexure; Sealed-Off Perforation of Carcinoma

H. S. (A-42-43), a female, 41 years of age, was admitted with the complaint of recurrent pain in the right lower abdomen for 10 days. She appeared to be acutely ill. Abdomen was distended and tender. A large, fluctuant mass was palpable in the right lower quadrant. Temperature was 101.6° F.; blood pressure, 110/72 mm. of Hg. The diagnosis of "twisted ovarian cyst" was made. Abdominal distention persisted. On one occasion the patient vomited a bile-stained fluid. Temperature rose to 105° F. Signs of peritonitis ensued. A flat roentgenogram of the abdomen showed marked gaseous distention of the right side of the colon and pneumoperitoneum. Supportive treatment was given. On the sixth day after admission the patient lapsed into coma and expired.

Necropsy Findings. On opening the abdominal cavity a tremendous amount of foul-smelling, purulent material gushed out under pressure. The serous surfaces of the abdominal organs were covered with fibrinous exudate. The proximal two-thirds of the colon down to about the splenic flexure was enormously ballooned out, and from there downward it was collapsed. The splenic flexure was the seat of a firm, annular tumor

mass which encircled the entire wall in napkin-ring fashion with hardly any free lumen discernible. Two minute perforations were seen above the tumor mass. The anterior surface of the cecum showed three longitudinal lentil-shaped tears of the serosa and the outer muscularis. The mucosa of the cecum was intact. Bauhin's valve was closed. Microscopically, there was separation of the muscular coats of the cecum, but no evidence of a pre-existing inflammation. The tumor was an infiltrating adenocarcinoma, grade 3, with invasion of the regional structures.

Anatomical Diagnosis. Infiltrating, stenosing adenocarcinoma of the splenic flexure of the colon with secondary perforation; diastatic tears of the cecum and ascending colon; fibrinopurulent peritonitis.

Case 4: Diastasis of the Cecum and Ileum; Taenial Tears of Cecum; Carcinoma of the Ascending Colon

G. T. (A-31-43), a female, 65 years old, was admitted with a 12 months' history of 15 recurrent attacks of abdominal pain, distention, and obstipation. Roentgenologic examinations of the intestines were negative. A barium enema "required 8 quarts of fluid to fill the colon." The patient appeared acutely ill, dehydrated, and pale. The abdomen was enormously distended, with intestinal coils being clearly visible. Temperature was 101° F.; pulse, 110; hemoglobin, 94 per cent; erythrocytes, 4.6 millions; leukocytes, 6200; neutrophils, 75 per cent. Diagnosis of "megacolon and intestinal obstruction" was made. At operation, performed the day following admission, the cecum was found to be enormously dilated and its taeniae torn. The cecum was incised and a catheter inserted. Two days later the patient expired.

Necropsy Findings. The cecum and most of the ileum were tremendously dilated. The distal half of the transverse colon and the descending colon were normal in caliber. The ascending colon, at a point 5 cm. above the cecum, was constricted, bottle-neck in appearance, and the seat of an annular, fungating carcinoma. The ileocecal valve was patent. A few serosal tears were noted within the terminal ileum. Microscopically, the cecum showed tears of the muscularis but no evidence of inflammation.

Anatomical Diagnosis. Stenosing adenocarcinoma of ascending colon; diastatic tears of cecum and small intestine; disseminated hemorrhagic ileitis and jejunitis.

Case 5: Diastatic Tears of Cecum and Ascending Colon Above Carcinoma of the Sigmoid

P. B. (A-160-44), a male, 51 years of age, was admitted in coma. On the previous day he had come home from work early because of severe pain in abdomen and chest. On admission he was unconscious, cyanotic, and dyspneic. Pulse was rapid and feeble. Abdomen was distended and tympanitic. Temperature was 102° F.; pulse, 120; respirations, 52; hemoglobin, 83 per cent; erythrocytes, 4.2 millions; leukocytes, 23,000; neutrophils, 69 per cent. Two hours later he expired. Diagnosis: "coronary thrombosis."

Necropsy Findings. The patient was a well nourished male of about 50 years. On opening the abdomen a small quantity of turbid fluid was found in the pelvis. The colon proximal to the sigmoid was ballooned out, while distal to it it was collapsed. The sigmoid was the seat of an annular carcinoma which occluded the entire lumen (Figs. 4 and 4, A). It measured 9 by 5 cm. and encircled the entire circumference of the intestine. On its anterolateral aspect there was a small perforation, 15 mm. in diameter, which was sealed off by adhesions. Within the greatly distended cecum and ascending colon were three diastatic tears spaced about 3 cm. apart. They were elliptoid, measured 30 by 10 mm., 32 by 12 mm., and 20 by 8 mm., respectively, and involved the serosa and muscularis only. The mucosa was intact. Microscopically, the diastatic areas showed no evidence of a pre-existing inflammation (Fig. 5). A few recently thrombosed veins were seen in one section (Fig. 6).

Anatomical Diagnosis. Annular, infiltrating, stenosing adenocarcinoma of the sigmoid with walled-off perforation; diastasis of the proximal portion of the colon with multiple tears of the cecum and ascending colon.

Case 6: Diastatic Rupture of the Rectosigmoid and Cecum Secondary to Congenital Atresia of the Rectum

J. G. (A-12-45), was a male infant, 1 week old. Abdominal distention was noted soon after birth. The anus was partly imperforate. Stools were fluid. A catheter introduced into the rectum could be passed for a distance of only 5 inches. Diagnosis: "intestinal obstruction due to constriction of rectum." At operation, the peritoneal cavity was found to be filled with meconium and feces. There was a wide perforation in the cecum. The patient died the following day.

Necropsy Findings. At necropsy the abdomen was found to be filled with fecal material. The rectum (Fig. 7) showed atresia with pseudoreduplication of its mucosa to form a blind pouch. The remainder of the colon was enormously dilated. Two diastatic perforations were found: one within the cecum, 25 by 10 mm., and another in the sigmoid, 30 by 10 mm. in size. Microscopically, there was a small area of organization at the site of the taenial tear (Fig. 8).

Anatomical Diagnosis. Congenital atresia of rectum with diastatic perforation of rectosigmoid and cecum.

Case 7: Diastatic Perforation of Descending Colon Secondary to Obstruction by a Stenosing Carcinoma of the Rectum

R. M. (A-34-46), a male, 50 years old, was admitted with a history of violent abdominal pain and vomiting for 7 hours and constipation for 2 weeks. He had had many operations for acute appendicitis, hernia, and fecal fistulae. On admission, he was in shock. Abdomen was rigid and tender, and liver dullness was obliterated. Temperature was 101° F.; pulse, 165; blood pressure, 40/0 mm. of Hg; hemoglobin, 90 per cent; erythrocytes, 4.6 millions; leukocytes, 7,000; neutrophils, 60 per cent.

Diagnosis: perforation of intestine with peritonitis. At operation, the abdomen was found to be filled with turbid fluid and fecal scybala. There was a huge rent in the descending colon. A transverse colostomy was performed. The patient expired 8 hours later.

Necropsy Findings. A tremendous amount of fecal matter filled the abdominal cavity at necropsy. A stenosing carcinoma was found in the rectosigmoid. A typical diastatic perforation was present 10 cm. above it (Fig. 9). It was elliptoid, 35 mm. long and 20 mm. wide, and situated near the mesenteric border. Microscopically, no evidence of inflammation was seen in the ruptured intestine. The tumor was a fairly well differentiated adenocarcinoma.

Anatomical Diagnosis. Infiltrating, stenosing adenocarcinoma of the rectosigmoid, with secondary diastatic rupture of the descending colon, 10 cm. proximal to the tumor; fecopurulent peritonitis; colostomy; chronic peptic ulcer of the duodenum; cloudy swelling of the viscera.

Case 8: Diastatic Tear of the Cecum and Ascending Colon Above a Stenosing Carcinoma of the Rectosigmoid

C. D. (A-37-46), a male, 62 years of age, was admitted with a 2-year history of constipation, loss of weight, diminution in size of stool, and vague lower abdominal pains. He was emaciated, had abdominal distention, ascites, and a mass in the left lower quadrant. Temperature was 99° F.; pulse, 100; hemoglobin, 95 per cent; erythrocytes, 4.3 millions; leukocytes, 7800; neutrophils, 64 per cent. Roentgenologic examination showed a constricting lesion in the rectosigmoid. At operation, a tumor in the sigmoid and widespread visceral metastases were found. A transverse colostomy was performed. The patient died 17 days later.

Necropsy Findings. The cecum and ascending colon, in spite of the previous colostomy, were still markedly distended at necropsy (Figs. 10 and 11). Their medial taenia, at a point 10 cm. from Bauhin's valve, was torn, the severed segments being 10 cm. apart. The partly stripped segment of the colon bulged through this rent. A stenosing carcinoma was present in the rectosigmoid. There were metastases in the liver, spleen, adrenal glands, and left ureter, with secondary hydronephrosis. Microscopically, no inflammatory changes were seen in the diastatic areas. The sigmoid showed an adenocarcinoma which invaded the entire wall.

Anatomical Diagnosis. Infiltrating, stenosing adenocarcinoma of rectosigmoid with metastases to liver, spleen, adrenal glands, left ureter, and regional lymph nodes; secondary hydronephrosis, left; diastatic tears of cecum and ascending colon.

Case 9: Multiple Stenosing Carcinoma of Sigmoid with Perforation; Diastatic Tear or Incomplete Perforation of Ascending Colon; Signs and Symptoms Chiefly on the Right Side

I. L. (A-55-46), a female, 38 years of age, was admitted with a 2-week history of abdominal pain. She had had a hemorrhoidectomy 2 weeks prior to admission. She was

well nourished and did not appear acutely ill. The abdomen was distended. There was tenderness and a mass in the right lower quadrant. Temperature was 103° F.; pulse, 100; respirations, 25; hemoglobin, 78 per cent; erythrocytes, 4.5 millions; leukocytes, 9000; neutrophils, 86 per cent. At operation, there was generalized peritonitis, with an abscess in the right lower quadrant. The source of peritonitis could not be determined. Antibiotics were given, but abdominal distention increased. On the 14th day following admission the patient expired.

Necropsy Findings. Two primary carcinomas were found in the sigmoid at necropsy, with perforation of the distal tumor and numerous diastatic tears of the ascending colon. Microscopically, no evidence of pre-existing inflammation was found in the diastatic intestine. No visceral metastases were present.

Anatomical Diagnosis. Two primary adenocarcinomas of sigmoid with localized perforation and multiple diastatic tears of ascending colon.

Case 10: Diastasis and Taenial Tears of the Cecum and Ascending Colon with Stenosing Annular Carcinoma of the Descending Colon

D. R. (A-414/49), was a male, 56 years old, who was admitted with a history of progressive abdominal distention for 7 days and obstipation for 3 days. He had had rectal pain after defecation for 5 years with occasional episodes of melena. On admission he was in acute distress: toxic, cachectic, dyspneic, and dehydrated. The abdomen was markedly distended. Barium enema showed an obstructive lesion in the sigmoid. The blood count was normal. He showed also a severe deformity of the spine. A hemorrhoidectomy was performed for large external and internal hemorrhoids. The patient refused to stay in the hospital. He was readmitted 11 days later in extremis. The abdomen was enormously distended, tympanitic, and tender. Temperature was 98.9° F.; pulse, 130; respirations, 50; blood pressure, 110/80 mm. of Hg; erythrocytes, 5.8 millions; leukocytes, 10,000; neutrophils, 94 per cent. Supportive treatment was given. The patient was taken to the operating room for surgical decompression, but expired before induction of anesthesia.

Necropsy Findings. At necropsy the body was cachectic and undernourished. The colon to the middle of the descending colon was enormously distended (Fig. 3). A moderate quantity of serosanguineous fluid was present in the abdomen. The serosa and anterior taenia of the cecum, ascending, and transverse colon were torn in places. The tears, four in number, were elliptical and averaged 2 by 1 cm. in size. The mucosa bulged through the torn muscle but was intact. The descending colon, at a point 16 cm. proximal to the anus, showed a bottle-neck-like constriction which on opening was the seat of a firm, annular carcinoma, 20 mm. in length. The tumor encircled the entire circumference of the intestine with almost complete occlusion of its lumen. Metastatic invasion of the regional lymph nodes and parietal peritoneum also was seen. Microscopically, no evidence of pre-existing inflammation was seen in the diastatic areas. The tumor was an adenocarcinoma, with lymphatic embolization.

Anatomical Diagnosis. Infiltrating, stenosing, annular adenocarcinoma of the descending colon, with secondary diastasis of the entire proximal colon and taenial tears of the cecum and ascending and transverse colon; acute peritonitis, early; metastatic invasion of the regional lymph nodes and peritoneum; Marie-Strümpell spondylitis.

Clinical Picture

The clinical picture is usually a combination of those caused by the primary stenosing lesion and by the secondary diastatic disease, and is chiefly that of intestinal obstruction. Quite frequently, however, the signs and symptoms brought about by the distended intestine are so pronounced that they overshadow those caused by the stenosing lesion. Since in the majority of cases there is a carcinoma in the left third of the colon, the symptom complex is frequently referred to the right side of the abdomen, where the diastatic disease develops. For this reason such conditions are frequently mistaken clinically for ovarian cysts, retrocecal appendicitis, or diseases of the gallbladder and liver. It is noteworthy that the diagnosis of intestinal obstruction is not made as frequently in these cases as might be expected.

A compilation of the clinical data from most of the cases reported in the literature as well as my own reveals the following salient features.*

Sex and Age. The sexes are about equally represented.† In early age the newborn alone is affected and usually in the first 10 days of life. The highest incidence is between the ages of 50 to 70 years with a considerable occurrence between the ages of 30 to 50.

Onset of Illness. As a rule, the symptoms of the diastatic syndrome are ushered in more or less suddenly. The majority of patients trace the onset of their illness to 1 to 12 days, and a few to several weeks or even months, before admission to the hospital.

Signs and Symptoms. Abdominal pain was present in 85 per cent of the cases. It was mostly colicky and intermittent. It might be generalized, left-sided, right-sided, or para-umbilical, the last two being the more frequent sites. Abdominal tenderness was present in 25 per

* The 81 cases which form the basis for this review are chiefly those which have been reported by the following authors: Corbin (1831), Baron (1836), Gogué (1844), Béraud (1847), Thurnam (1848), Pretty (1851), Luke and Gibbon (1856), Markham (1857), Hayden (1869), Lépine (1870), Wernich (1870), Anger (1872), Dowse (1873), Holmer (1874), Morris (1879), Goodhart (1879), Mackenzie (1879), Heschl (1880), Marshall (1880), Müller (1884), Wharton (1890), Begemann (1891), Cullingworth (1897), Albarran and Lavillauroy (1898), Jacomet (1898), Kocher (1898), Schwob (1898), von Beck (1898), Gandy and Bufnoir (1899), Luys (1899), Létulle (1899), Morestin (1900), Anschütz (1902), Sauer (1902), Maleyx (1912), Kreuter (1919), Guillaume (1922), van Beuren (1923), Russell (1928), Riche and Guibal (1929), Medearis (1933), Wakeley (1934), Saeltzer and Rhodes (1935), Ravid (1950).

† However, of the 10 cases observed by me, 8 were male and 2 female.

cent of the cases. Abdominal rigidity and muscle spasm were present in 16 per cent of the cases and were mostly right-sided. Palpable mass was present in only 8 per cent. Visible peristalsis was rather infrequent in the entire series. Constipation was by far the most constant single symptom encountered. Vomiting was present in 75 per cent of the cases. It was either bilious or fecal. Melena was present in only 13 per cent. Alcoholism or dietary indiscretion with vomiting was encountered in cases of diastatic perforation of the stomach and esophagus. Roentgenologic examination showed marked distention of a portion of the colon, and occasionally of the small intestine as well. Pneumoperitoneum was sometimes seen. Barium enema studies, a procedure of doubtful safety in such cases, sometimes revealed the site of the primary stenosing lesion.

Clinical Forms. The disease may manifest itself in either of two forms: (1) an acute fulminating type, in which the signs and symptoms appear suddenly and in rapid succession; (2) a subacute or chronic type, in which the signs and symptoms extend over a period of weeks or months culminating in an acute abdominal catastrophe or coma.

Course. The disease, when unrecognized, usually progresses in a dramatically rapid course and terminates fatally within a few days or weeks. In rare cases, the disease may even last for months (van Beuren, 1923). However, when the diastatic condition is recognized early enough and operative interference is instituted promptly, there may be recovery.

Pathologic Features

Pathologic Features of the Primary Obstructive Lesion

Type of Obstructive Lesion. As seen from Table I, which is a compilation of the 81 cases collected from the literature, including 10 reported here, the predominating lesion by far is an intrinsic, stenosing carcinoma which is most frequently situated in the distal or left third of the colon (about 62 per cent). Peritoneal adhesions were the second most frequent cause of intestinal obstruction (13.5 per cent). These were either old fibrous or fresh fibrinous adhesions. Congenital anomalies ranked third in frequency (7.4 per cent), and consisted chiefly of atresia of the rectum or imperforate anus. In 5 cases (6 per cent) the obstruction was extrinsic in the form of a carcinoma of the ovary, metastatic lymph nodes, myoma of the uterus, and an echinococcus cyst of the liver. Several cases of lymphogranulomatous stricture as the cause of "spontaneous rupture" of the colon were reported also (Lichtenstein; Pollard and Hellendall). Volvulus was present in 2 cases. In diastatic perforation of the small intestine the primary lesions were

chiefly adhesions and extrinsic tumors. In the cases of "spontaneous" rupture of the stomach, which were reported in the literature up to 1939, some of the causes were as follows: stenosis of the duodenum, carcinoma of the pylorus, carcinoma of the esophagus, adhesive bands, incarceration of stomach in a diaphragmatic hernia, vomiting or straining during labor, alcoholism and overloading of stomach with food, and ingestion of sodium bicarbonate (Lemmon and Paschal).

TABLE I

Type of Obstructive Lesion

	Number of cases	Percentage
Carcinoma, intrinsic	50	61.7
Carcinoma, extrinsic	3	3.7
Tumors, benign	1	1.2
Adhesions	11	13.5
Congenital anomalies	6	7.4
Volvulus	1	1.3
Not stated	5	6.2
Undetermined	3	3.7
"No lesion"	1	1.2
	Total 81	

Site of Obstruction. As seen from Table II, in as many as 55 cases of the large intestine (76 per cent), the obstruction was situated in the left third of the colon. Carcinoma was by far the most frequent cause, having occurred in 59 per cent of the cases. The most frequent site of carcinoma in this series was in the sigmoid colon.

TABLE II

Sites of Obstruction

Sites	Number of cases	Percentage
Large intestine		
Sigmoid	32	44.4
Rectum	10	13.8
Splenic flexure	9	12.5
Hepatic flexure	7	9.1
Descending colon	4	5.5
Ascending colon	2	2.7
Transverse colon	2	2.7
Site not given	2	2.7
Small intestine	4	5.5
	Total 72	

Anatomical Features. The macroscopic as well as the microscopic pictures of the stenosing lesions did not differ from those which were not associated with diastasis; hence description may be omitted here. It may be added, however, that frequently there was some hypertrophy of the muscular coat of the intestine immediately above the site of obstruction, an observation already made by Haussmann in 1882.

Pathologic Features of the Secondary or Diastatic Lesion

Site of Maximum Distention. As seen from Table III, the most frequent site of diastatic tearing or perforation of the colon was in its right third (80.8 per cent). Of the 78 cases of intestinal perforation reported, 53.8 per cent occurred in the cecum, 15.3 per cent in the ascending colon, 8.9 per cent in the transverse colon, and 5.1 per cent in the descending colon. In only one case was the hepatic flexure the seat of perforation. Altogether, 10 cases of diastatic perforation of the small intestine were reported (12.8 per cent). They were situated in different segments of the ileum, jejunum, and duodenum. In the cases of "spontaneous" rupture of the stomach, the most frequent site of tear was in the lesser curvature, with the anterior portion of the stomach being the next most common site (Lemmon and Paschal). In "spontaneous" rupture of the esophagus the lower end near the cardia was most commonly involved (Eliason and Welty; Walker).

TABLE III

Sites of Maximal Distention or Perforation

Sites	Number of cases	Percentage
Cecum	42	53.8
Ascending colon	12	15.3
Transverse colon	7	8.9
Descending colon	4	5.1
Sigmoid colon	2	2.4
Hepatic flexure	1	1.2
Small intestine	10	12.8
	Total 78	
Right third of colon	55	80.8
Middle third of colon	5	8.8
Left third of colon	7	10.3

Gross Appearance of the Site of Diastatic Tear or Perforation. The characteristic diastatic lesion is a sharp, elliptic or lentil-shaped tear varying from 5 to 40 mm. or more in length, and with its longer diameter usually running parallel to the longitudinal axis of the intestine. The borders are thin, clean-cut, and usually show no evidence of previous inflammation. In the small intestine the tears are usually situated at the mesenteric border which is the place of least resistance. In the colon, perforation usually takes place between the anterior and posterior taeniae and chiefly on the anterior surface. The anterior taenia is most commonly involved. The tear in the outer muscularis is usually wider than in the inner circular muscle layer so that the latter often overhangs the opening to a greater degree than the former. There may be multiple tears in the same segment. The area of rupture is invariably the most distended portion of the intestine. When such a case is oper-

ated upon and the intestine is observed before rupture has taken place, a small tear may be seen in the serosa or taenia. Usually, however, such a tear may be unnoticeable, as it is by far overshadowed by the enormous distention of the intestine. As frequently happens, while manipulation of the distended intestine is in progress, its weakest dilated segment suddenly ruptures. The distended intestine does not invariably perforate. Thus in 5 of my cases and in those of Luys, Létulle, Bouveret, and van Beuren the intestine was enormously distended, but remained intact.

Microscopic Appearance of the Diastatic Intestine. Sections from the areas of diastatic rupture usually showed evidence of long-standing atrophy of all layers with only a minimal degree of edema or round cell infiltration. Occasionally, small healed scars might be seen in areas where the muscularis had been previously torn (Figs. 2, A and 8). There was usually no evidence of a pre-existing inflammation (Figs. 2, 5, and 6). This fact has been pointed out by the earlier observers. Thus Heschl stated that there was no edema or inflammation of the affected part. Létulle observed that the mucosa did not show a trace of acute or chronic inflammation at any site of perforation. Maleyx stated emphatically that there need not be an ulceration in order to cause a perforation. Occasionally, however, and as seen in one of my cases (Fig. 6), a few thrombosed vessels may be found in the vicinity of the ruptured taeniae.

Mechanism and Pathogenesis

Among the theories which have been expounded to explain the mechanism of perforation in diastatic conditions of the gastro-intestinal tract, three require discussion.

The Vascular Theory. The vascular theory as expounded by Kocher, Guillaume, van Beuren, and others, attempts to explain the intestinal perforation on a purely vascular basis. When the lumen of the intestine distends—so claim these observers—the blood vessels within its wall become obliterated. This, according to van Beuren, affects the veins first, and is later followed by extravasation of blood within the submucosa. The pressure ultimately occludes the arteries as well, and the area supplied by these vessels becomes necrotic. A hemorrhagic infarction is thus produced which in turn is followed by perforation. Van Beuren also stated that in his cases of cecal rupture the tear was situated on the antimesenteric surface close to the site of obstruction, where the anterior and posterior cecal arteries anastomose. Guillaume found only here and there a vessel which was altered and on the way to obliteration.

The Toxic-Infectious Theory. In a closed loop of the intestine—it is claimed by the advocates of the toxic-infectious theory—there is an

increase in the virulence of the bacterial flora, an increased permeability of the intestinal wall, and invasion of the mucosa, with subsequent necrosis, ulceration and extension of the process to the other layers of the wall. The same effect may be produced by a fecal mass which acts as a foreign body and causes mucosal ulceration.

The Mechanistic Theory. The mechanistic theory explains the "perforation à distance" as being due chiefly to the progressive distention of the intestine, and is in the nature of a "blow-out" tear, the same as obtains in any hollow object that is constantly distended with air or gas, and which bursts as soon as the intraluminal pressure exceeds the tensile strength of its wall. It is akin to the bursting of a fluid-filled bleb of the skin in an edematous subject, of the inner diploe in progressive hydrocephalus, or of an empyema necessitatis. This theory is supported by the following facts: (1) As observed in almost all of the cases, no gross or microscopic evidence of a pre-existing mucosal inflammation or ulceration could be found in the distended or ruptured loops of the intestine. (2) The gross appearance of the tear is that of a fresh, clean-cut wound as seen in any "blow-out" perforation which is produced mechanically. (3) As seen from the distention experiments performed upon various parts of the gastro-intestinal tract in which air or water was used, the general appearance of the rupture thus produced is similar in almost every respect to that observed clinically. Not only are the tears almost identical in general outline and configuration, but their location, distribution, and anatomical relationship with respect to the mesentery and other layers are also almost the same. Thus, in the experimental diastatic perforation* it is also the cecum or ascending colon which is the most frequent site of tearing or perforation. The latter is likewise usually situated at the antimesenteric border of the colon. First to tear are invariably the taeniae and then the longitudinal and circular muscle layers, with bulging of the mucosa taking place last, exactly as observed in the clinical cases. In experiments on the small intestine, rupture occurs along the mesenteric border and is also of the same appearance as seen clinically.

These facts necessarily imply that the segment of the intestine which ruptures in diastasis above an obstruction is more or less normal. Thus, to the question whether a normal intestine can rupture, the reply must be in the affirmative, although such a seemingly radical view may at first appear to be contrary to the usual teaching. This mechanistic view was shared by many early writers. Thus, Corbin, as early as 1831, in his

* Seen by me and by other observers previously. A detailed report of the experimental aspects of this problem will be given in a forthcoming publication. The appearance of an experimentally produced diastatic perforation of the cecum may be seen in Figure 12.

graphic description of the oval perforation with neat border as though cut by a punch, stated that the stenosis was the cause of the perforation. Pretty (1851) even produced microscopic evidence that the opening "was free from inflammation." Likewise Markham (1857) expressed the opinion that "the rupture of the gut evidently resulted from its distention by the air within it." It was Heschl, in 1880, who was the first to expound this mechanistic theory clearly. He stated emphatically that there was no trace of an ulcer in the intestine, no edema or infiltration of the affected part, and that it must have been caused by congested feces and perhaps aided by the gas. He also observed that the compression of the intestinal layers prevented hemorrhages within the distended intestine.

The mechanical factor was further emphasized by Anschütz (1902), who explained the predilection of the cecum as the seat of diastatic perforation by the fact that it has the widest caliber and the greatest surface for a given length, and, therefore, in accordance with the physical law of transmission of pressure, suffers the greatest amount of pressure and consequently ruptures first. Maleyx (1912) held the same view and emphasized that no antecedent ulceration of the mucosa was necessary in order for such a perforation to take place. Wangensteen (1942) also was of the opinion that the mechanistic theory provides a better explanation for the end results of intestinal obstruction than does the theory of toxic absorption.

In the cases of "spontaneous" perforation of the stomach and esophagus which were reported in the literature (Beal; Eliason and Welty; Klein and Grossman; Walker), it was also the normal portion of the stomach or esophagus which ruptured. The same principle of increased intraluminal pressure operated in most of these cases as well, and was probably aggravated by pylorospasm and cardiospasm.

The Ileocecal Valve

The most important single factor in diastatic rupture of the colon is the condition of Bauhin's valve. When this valve is incompetent, the accumulated fluid and gas within the colon will regurgitate into the ileum and the pressure effect on the cecum temporarily will be relieved. This will explain the fact that in a goodly number of cases of intestinal obstruction with a patent ileocecal valve the colon may not perforate at all. However, because of increase in back pressure, perforation of the intestine will ultimately take place, but this will occur not in the colon but in the small intestine, even as high as the duodenum. On the other hand, when the ileocecal valve is closed or competent, a closed loop obstruction will be formed, and the accumulated fluid and gas

must sooner or later be liberated by bursting of the colon at its weakest point above the obstruction.

The important rôle that the ileocecal valve plays in diastatic perforation of the obstructed colon was emphasized also by Anschütz, Sperling, Wangensteen, Wakefield and Friedell, and others. The frequency of incompetence of the ileocecal valve is usually given as between 50 to 60 per cent (Ravid; Shanks, Kerley, and Twining; Wakefield and Friedell). This high incidence of incompetence of the ileocecal valve explains the fact that diastatic perforation of the colon is a relatively infrequent occurrence.*

In the cases of "spontaneous" rupture of the stomach and esophagus which have been reported in the literature, pylorospasm or cardiospasm undoubtedly played a rôle similar to that of Bauhin's valve.

In conclusion, it would appear that no one single theory can explain all diastatic perforations. However, on critical review of most of the cases reported in the literature, and in light of the early and recent experimental work done on this problem, it would appear that in the great majority of cases the purely mechanical factor is sufficient to explain the entire train of events leading to perforation. In a certain number of cases, however, additional explanation may be sought in factors of circulatory or of toxic-infectious nature.

Summary and Conclusions

The clinicopathologic syndrome of "diastasis" and "diastatic perforation" of the gastro-intestinal tract concerns itself with a train of signs, symptoms, and pathologic changes which take place when a more or less normal segment undergoes considerable distention, as a result of the presence of an intrinsic or extrinsic obstructive lesion within another distally situated segment of the intestine. The secondarily affected loop of the intestine thus first suffers tearing of its serosal and muscular coats and ultimately perforates.

The diagnosis of this condition may be missed at operation or autopsy, for it may be misinterpreted as decubital ulcer, stercoral abscess, or perityphlitis.

In the majority of cases the diastatic process involves the right side of the colon, since the primary obstructive lesion is most frequently situated in the left side of the colon.

* Carman, however, stated that in his barium enema studies of the colon the barium passed into the ileum in about 90 per cent of cases. This higher incidence of incompetence of the valve may be explained by the fact that in introducing barium into the colon manual force is usually employed and a much higher hydraulic pressure is created than that which obtains in the obstructed intestine.

The primary stenosing lesion in the intestine can be an intrinsic tumor, congenital atresia, a chronic granuloma, or an extrinsic lesion in the form of adhesive bands, tumors, or cysts.

Among the chief primary exciting causes in the diastatic perforation of the stomach are the following: stenosis of the duodenum or the pylorus, tumors of the esophagus, incarceration of the stomach in a diaphragmatic hernia, adhesive bands, vomiting and straining during labor, overloading of stomach with food or alcohol, and ingestion of sodium bicarbonate.

The proximal third of the colon, and particularly the cecum, is the most common site of the diastatic process. The part of the colon involved shows first a splitting of the taenia, then a longitudinal tear of the outer muscularis, followed by a circular tear of the inner muscle, and finally perforation of the mucosa. No evidence of pre-existing intrinsic disease of the ruptured diastatic intestine or stomach is to be found.

The ileocecal valve plays an important rôle in the diastatic process. When it is patent, no perforation of the colon will take place. When it is closed, a closed-loop obstruction will be formed, and the distention, unless relieved in time, will lead to perforation. Rarely, when the obstruction is within the cecum itself, the distention may be transmitted to the ileum. In "spontaneous" perforation of the stomach and esophagus, the pyloric and cardiac sphincters play a rôle similar to that of Bauhin's valve.

Diastasis and perforation are best explained on a mechanical basis, namely, by the increase in the intraluminal hydraulic pressure exerted by the accumulated matter and gas, which exceeds the tensile strength of the wall. Toxic-infectious and vascular factors may, at times, also contribute to the diastatic process.

In distention experiments on the stomach and intestine, the sites and general character of the tears resemble very closely those observed clinically.

Only in better understanding and early recognition of the diastatic syndrome lies the solution to the possible cure of both the primary obstructive and the secondary diastatic disease.

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DESCRIPTION OF PLATES

PLATE 9

FIG. 1. Case 1. Perforated cecum and ascending colon. (Photograph of formalin-fixed specimen.) At each of the upper and lower borders of the gaping defect can be seen the torn segments of the anterior taenia.

FIG. 2. Case 1. Photomicrograph of specimen seen in Figure 1, across the ruptured taenia. Mucosa is normal. The torn segments of the muscularis can be seen on each end of the lower border. There is edema of the midportion. No evidence of inflammation present. × 26.

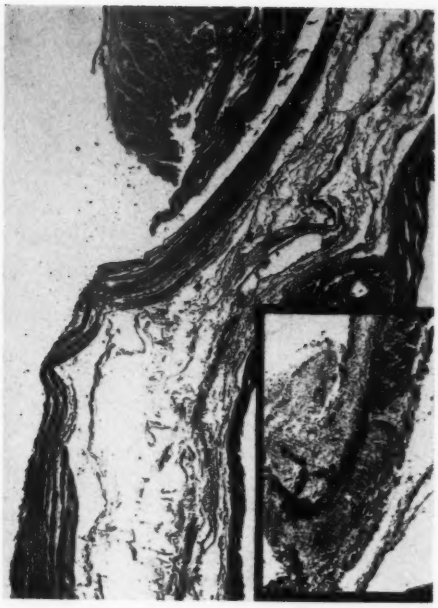
FIG. 2, A (insert). Case 1. Photomicrograph of the lower portion of the cecum, at the site of a torn taenia. There is a small wedge-shaped area within the muscularis and serosa which is the site of a previous tear. It contains some newly formed capillaries and a loose fibrillar connective tissue. × 21.

FIG. 3. Case 10. Photograph of the large and small intestines. A stenosing carcinoma (partly opened) is seen at the right lower border. The colon above it is enormously distended. Four taenial tears can be seen along the cecum, ascending, transverse, and descending colon.

FIG. 4 (insert). Case 5. Photograph of the entire colon. On the right lower border is a stenosing carcinoma in the rectosigmoid, with a walled-off perforation. On the left are the distended cecum and ascending colon. Between the two lower arrow marks is a typical diastatic perforation with the torn segments of the taenia clearly visible.

FIG. 4, A. Case 5. A higher magnification of Figure 4, showing the torn taenia and the elliptoid tears of the muscularis.

1



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Diastasis of Gastro-intestinal Tract

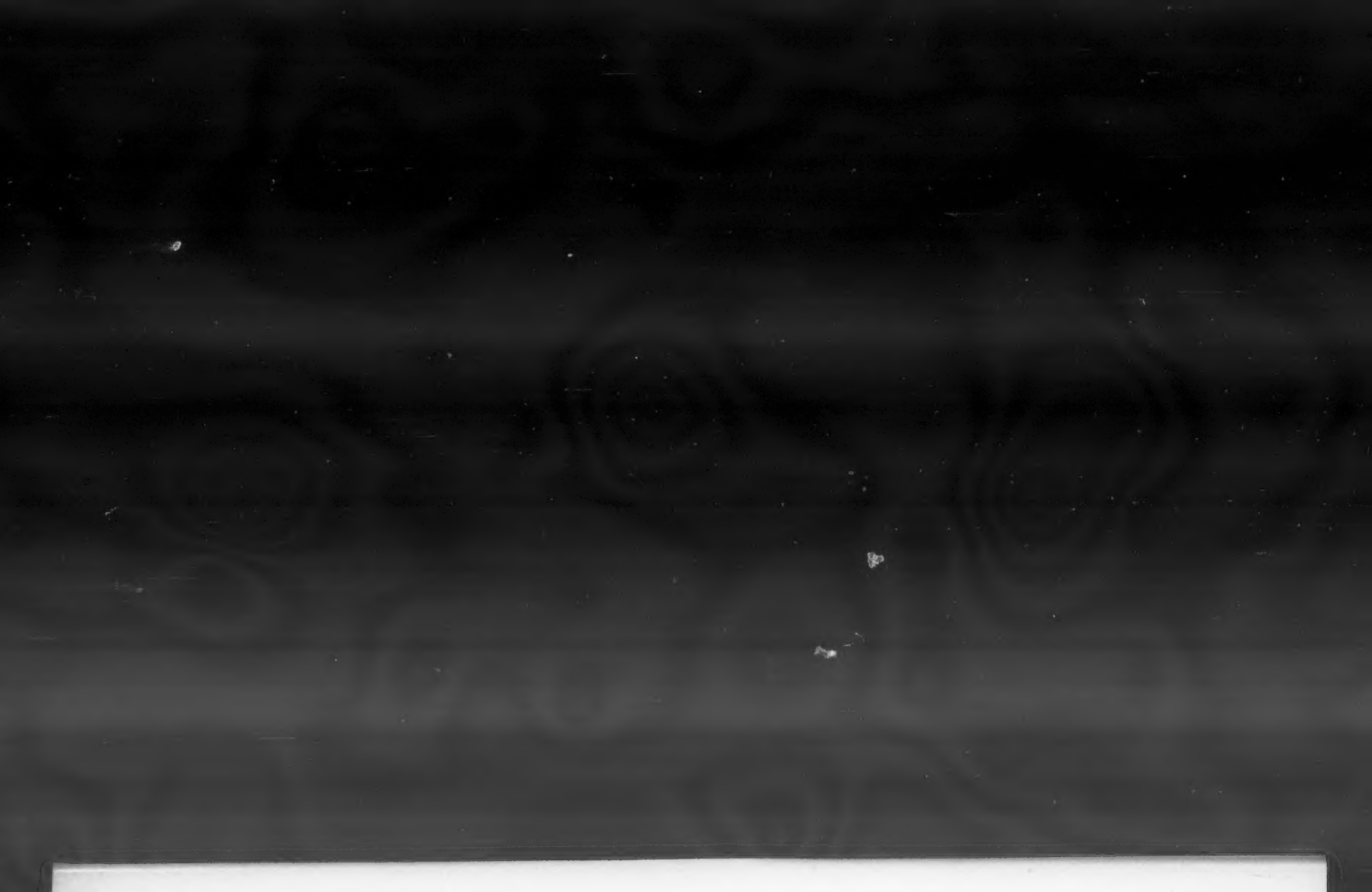
Plate 10

Fig. 5. Case 5. Photomicrograph of section taken at the site of the torn taenia. As in the other cases, the mucosa is intact. The torn segments of the muscularis may be seen on the right. No evidence of inflammation is present. × 13.

Fig. 6. Case 5. Photomicrograph from another portion of the ascending colon. The mucosa is intact. The torn muscularis is seen. Several thrombotic veins are found in the midportion. × 22.

Fig. 7. Case 6. Congenital atresia of rectum. (Organs *in situ;* right thigh on left). Left probe inserted in the atretic rectum. Upper portion of rectum and ascending colon are greatly distended and show diastatic tears.

Fig. 8. Case 6. Photomicrograph at site of cecal tears. The mucosa shows no evidence of inflammation. There is a recent separation of muscle bundles with an area of organization. × 32.



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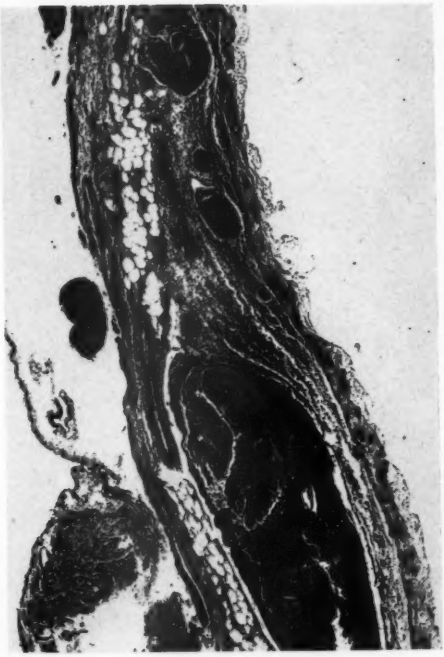

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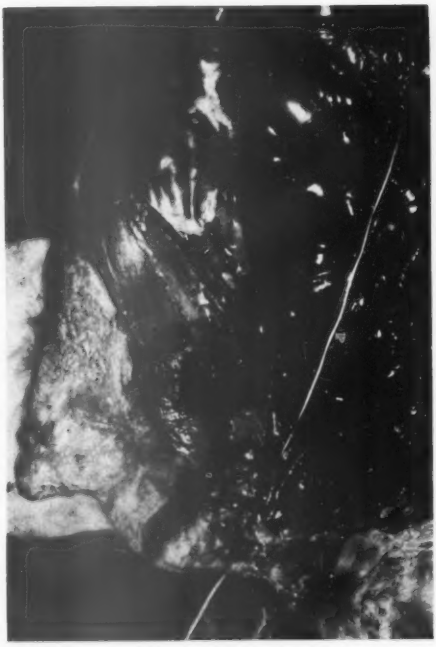

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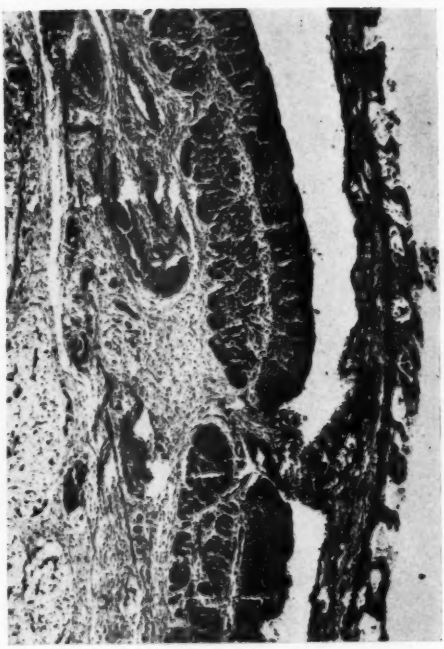

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Diastasis of Gastro-intestinal Tract

PLATE 11

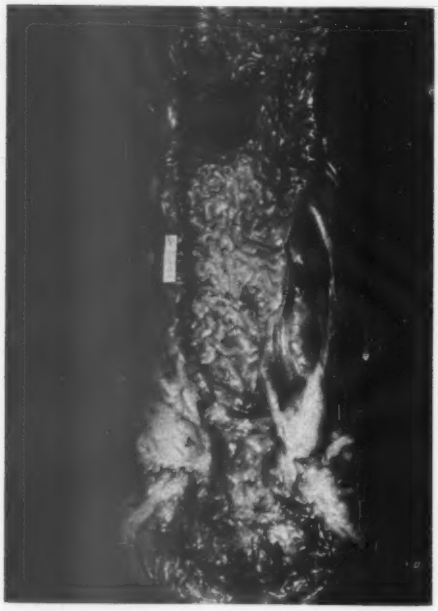
FIG. 9. Case 7. Below is the rectum with a fungating carcinoma. At the junction of the middle and upper thirds is a wide defect which is the site of diastatic perforation of the midportion of the descending colon.

FIG. 10. Case 8. Photograph of the entire colon. On the right, is the opened descending colon with an infiltrating carcinoma of the rectosigmoid (arrow). On the left is the enormously distended cecum and ascending colon. The arrow indicates the diastatic tear of the serosa and taenia. The liver shows diffuse metastatic invasion. The kidney shows hydronephrosis, secondary to obstruction of its ureter by lymph nodes containing metastases (bottom of picture).

FIG. 11. Case 8. Higher magnification of Figure 10, showing the typical diastatic tear of the ascending colon.

FIG. 12. Appearance of the cecum and ascending colon with a diastatic rupture of the colon produced experimentally in a post-mortem human specimen. It shows three typical lentiform diastatic tears, with splitting of the taenia of the cecum and ascending colon, which are practically identical with those observed clinically.

9



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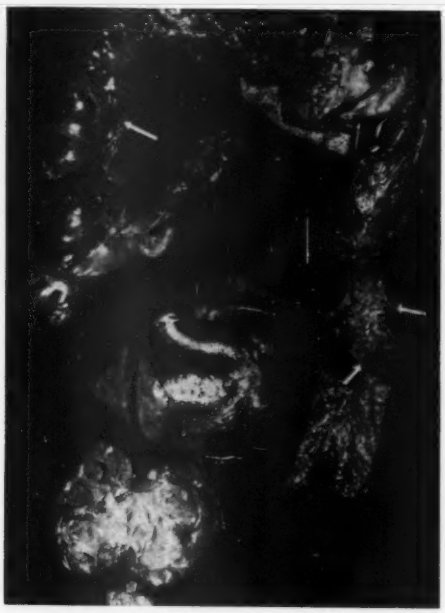

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Ravid

Diastasis of Gastro-intestinal Tract

THE EFFECT OF HEPBISUL (HEPTYL ALDEHYDE-SODIUM BISULFITE ADDITION COMPOUND) AND THYROXIN ON WALKER RAT CARCINOMA 256 *

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During the investigation of the effects of flavoring oils when added to the diet of a high mammary cancer strain of mice, Strong [1] noted that true oil of gaultheria not only delayed the appearance of the tumors but also prolonged the life of the animals after the growths became apparent. Although true oil of gaultheria is more than 95 per cent methyl salicylate, synthetic oil of wintergreen (methyl salicylate) had very little effect on the tumors.[2,3] Serial distillation of true oil of gaultheria yielded a high boiling point fraction that produced no changes in the tumors, and a low boiling point fraction (distilling over below the boiling point of methyl salicylate) that contained the active principle.[4,5] The latter was more effective than true oil of gaultheria, for when tested on animals it showed an increase in survival time, an increase in softening, liquefaction, and necrosis of the growths, and complete regression of 4 of 34 tumors.

Assuming that heptyl aldehyde was an ingredient of the low boiling point fraction, it was tested and was found to be effective, but to a lesser degree than the active principle itself.[6,7] Heptyl aldehyde, however, had two unfavorable properties—it deteriorated rapidly and it produced ulceration at the site of the injection. The addition of synthetic methyl salicylate protected heptyl aldehyde from oxidation and the combination was actually more effective than was heptyl aldehyde alone, producing complete regression of the tumors in 12 of 50 animals used.[6,8] The new mixture, however, did not decrease the local irritative properties upon injection. Subsequently, Strong discovered that the addition of sodium bisulfite to heptyl aldehyde protected the latter from deterioration, practically neutralized its harmful action at the site of injection, and had a definite salutary effect upon the tumors.[9,10]

Following the work of Strong, several experimenters tested the tumor inhibitory property of heptyl aldehyde with mixed, but generally speaking, unfavorable results. Thus Baumann, Kline, and Rusch,[11] in 1938, administered heptyl aldehyde in food and subcutaneously to mice with (1) spontaneous mammary adenocarcinoma (strain A mice), (2) pri-

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mary aural tumor induced by ultraviolet light, (3) primary epithelial tumor induced by painting with benzpyrene, (4) primary sarcoma induced by the subcutaneous injection of benzpyrene, and (5) transplanted spindle cell sarcoma originally induced by benzpyrene. They confirmed the local irritative effect of heptyl aldehyde when used subcutaneously and stated that feeding the drug failed not only to prolong the life of the tumor-bearing animals but also to alter the character of the tumor. They concluded that heptyl aldehyde was not a universal tumor inhibitor.

Clark,[12] in 1939, administered heptyl aldehyde by stomach tube, in food, drinking water, and by injection directly into the tumors of rats bearing a subcutaneously transplanted spontaneous sarcoma that originated in the liver of a female rat. The treated animals showed no greater regression, no greater liquefaction, and no increase in survival time over the controls. Willmer and Wallersteiner,[13] in 1939, found that heptyl aldehyde inhibited the growth of chick periosteal fibroblasts growing *in vitro*. Boyland,[14] in 1940, reported no inhibition of the Crocker sarcoma 180 by feeding 50 mg. of heptyl aldehyde per day. He did, however, obtain 14 per cent inhibition of tumors induced by the injection of 1 mg. of methylcholanthrene in stock mice, and in spontaneous mammary tumors of mice. Garai,[15] in 1941, administered heptyl aldehyde-sodium bisulfite intraperitoneally and subcutaneously to mammary-tumor-bearing mice of the C3H, Dba and Paris R3 strains. He noted that the drug exerted a destructive effect on the kidneys and liver which far outweighed the partial retardation of the growth of the spontaneous tumors. He concluded, therefore, that it was not a suitable chemotherapeutic agent for cancer.

In a general review of cancer therapy, Woglom,[16] in 1947, stated that at first heptyl aldehyde seemed to cause the regression of 25 per cent of mammary carcinomas in mice, but that further observations did not confirm these findings, and that subsequently interest in the compound appeared to have been lost. In a discussion of Woglom's remarks, Strong stated that his work with heptyl aldehyde had been verified by regressions of tumors in both Switzerland and in England, that the effect of liquefaction lies in a very narrow range of dosage, and that the animals must be put on a strictly quantitative basis. He further stated that heptyl aldehyde of itself would not do enough but that some other chemical combined with heptyl aldehyde would continue the process of control farther than any compound then known to science.

Our interest in heptyl aldehyde and heptyl aldehyde-sodium bisulfite addition compound dates to the work of Strong. We have worked exclusively with transplantable tumors. Formerly we used the Crocker

mouse sarcoma 180, rat sarcoma 39, and Walker rat carcinoma 256. More recently we have been using only the Walker rat carcinoma 256 because (1) the tumor takes in almost 100 per cent of cases, (2) it is attended by less than 0.6 per cent spontaneous regressions, (3) it kills the host in almost 100 per cent of cases, (4) it is vigorous in its growth and is not readily influenced by factors that often retard growth of other transplantable tumors, and (5) the viable portion of the tumor in control animals remains remarkably uniform, thus making alterations induced by chemicals easily recognizable. Although we have had some success with the heptyl aldehyde-sodium bisulfite addition compound alone, its effect upon the tumors was slight, and we therefore began casting about for a possible catalytic agent. This we found in natural thyroxin.* By administering a combination of the two compounds to Sprague-Dawley rats bearing the Walker carcinoma 256 we have been able to produce complete regression of the tumors in one-fourth of the animals treated. These results, we feel, justify the following report.

Materials and Method

Chemicals

The chemicals used in this experiment, except as otherwise indicated, were prepared at the Chemical Unit of the Elizabeth Storck Kraemer Memorial Foundation located at the du Pont Experimental Station on the Brandywine, Delaware. Since the term heptyl aldehyde-sodium bisulfite addition compound and the alternative term sodium alpha-hydroxy heptane sulfonate are cumbersome, the word "hepbisul" has been coined to designate this agent and will be used henceforth in this presentation. The empirical formula for this compound is $C_7H_{15}O_4SNa$ and its structural formula is $CH_3CH_2CH_2CH_2CH_2CH_2CH(OH)SO_3Na$. It was prepared by adding freshly distilled heptyl aldehyde (obtained from the Baker Castor Oil Company) to a hot concentrated aqueous solution of sodium bisulfite, with stirring. After the reaction mass cooled, alcohol was added to complete the precipitation. The solid was filtered, washed with alcohol, and dried in air. Aqueous solutions of the powder in desired strengths were put up in ampules, sealed, and then sterilized using 12 lbs. of pressure for 25 minutes. The material was thus suitable for parenteral use.

Two types of thyroxin were used: synthetic and natural. The synthetic compound (Roche-Organon) was obtained already prepared in ampules for parenteral administration and was used unaltered. Natural

* The theoretic basis for selecting thyroxin was the possibility of stimulating the neoplastic cells to more rapid proliferation and thus rendering them more vulnerable to heptyl aldehyde-sodium bisulfite.

thyroxin was obtained in the form of crystals from E. R. Squibb and Sons. The crystals were dissolved in a small amount of 0.1 N sodium hydroxide, diluted with distilled water to the desired concentration, put in ampules, and then sterilized. An attempt was made to combine hepbisul and thyroxin in a single ampule, but the effort was doomed to failure because the degree of alkalinity required to keep thyroxin in solution is sufficient to cause destruction of hepbisul. The latter is unstable in a pH much below or above 7.0.

Animals and Tumors

The animals used in this experiment were the Sprague-Dawley strain of rats. Originally one set of animals was obtained from Memorial Hospital, New York City, while 4 years ago another set was obtained from Sprague-Dawley, Inc., Madison, Wisconsin. Since then they have been inbred (brother-sister mating) in our own laboratory. The diet consisted of purina dog chow with a small amount of buckwheat added. Lettuce was given once a week and the animals had access to water at all times.

The transplantable tumor—Walker rat mammary carcinoma 256—was obtained from the Crocker Laboratory, Columbia University, New York City, about 1936. The technic of transplantation into the axilla was carried out under aseptic conditions and was standard except that forceps were used instead of a trochar. Animals of 1½ to 2 months of age and weighing about 150 gm. served as hosts and the donor rat containing the transplanted tumor was killed on the seventh to the tenth day after transplantation.

Experiment

In testing the chemicals for tumor inhibitory activity the animals were used 1 week after the tumor was transplanted. They were paired so that the tumors in the control group were of approximately the same size as those in the treated group. Animals bearing tumors that measured less than 15 by 10 by 5 mm. were not used. Subcutaneous injections of hepbisul and thyroxin were administered simultaneously (but not mixed) in the flank opposite the tumor on the seventh and each subsequent day after transplantation except Sunday. The animals were weighed every other day and the dosage was calculated accordingly. The tumors were measured in three dimensions at weekly intervals. Animals bearing tumors that showed no response to the chemicals (and their corresponding controls) were killed 21 days after transplantation, but those in which the tumor was small and regressing were allowed to live until the growth completely disappeared or until it was apparent

that it would regress no further. At necropsy the major organs, particularly the liver and kidneys, were carefully inspected and, from animals selected at random, liver and kidney were taken for histologic examination. All tumors were inspected grossly and examined histologically. Tissues were fixed in 10 per cent formalin and stained routinely with hematoxylin and eosin. In selected cases Masson's stain and sudan II were used also.

After preliminary trials the optimum dosage arrived at and thereafter used throughout the experiment, was 30 mg. of hepbisul per 100 gm. of body weight and 0.052 mg. of thyroxin per 100 gm. of body weight. The control animals received an equivalent amount (in cc.) of normal saline solution. In the experiment, 108 animals were given both hepbisul and natural thyroxin and 108 animals were used as controls. In addition, 50 animals received hepbisul alone; 50, natural thyroxin alone; 50, hepbisul and synthetic thyroxin; and 50, normal saline solution. Each of the groups of 108 and 50 animals, both treated and controls, was composed of an equal number of males and females.

Results

The results of the experiment are outlined in Table I. Under the heading of "favorable response" are listed those growths that showed varying degrees of degeneration, necrosis, and fibrosis of the neoplastic tissue but in which viable neoplastic cells were still apparent. Of the animals that received hepbisul and natural thyroxin, histologic examination of the tumor area disclosed no tumor cells in 27 cases (14 males and 13 females) and a favorable response in 12 (8 males and 4 females), making a total of 39 animals or 36 per cent in which the drug had an effect upon the tumor. Of the corresponding 108 animals that received normal saline solution, one showed complete regression and no others showed a favorable response. Of the 50 animals that received hepbisul alone, 4 showed complete regression of the tumor and none showed a favorable response otherwise, making a total of 4, or 8 per cent, favorable reaction.

Table I

Summary of Experimental Data Using Hepbisul, Natural Thyroxin, and Synthetic Thyroxin, Alone and in Combination, on Rats Bearing the Walker Carcinoma 256

Treatment	Number of animals	Complete regression	Favorable response	Total
Hepbisul and natural thyroxin	108	27	12	39 or 36%
Saline solution	108	1	0	1 or 0.9%
Hepbisul	50	4	0	4 or 8%
Natural thyroxin	50	1	0	1 or 2%
Hepbisul and synthetic thyroxin	50	2	0	2 or 4%
Saline solution	50	0	0	0

Of the 50 animals that received natural thyroxin, only one showed complete regression of the tumor and no others showed a favorable response, making a total of one, or 2 per cent, that showed a favorable effect. Of the 50 animals that received hepbisul and synthetic thyroxin, in 2 there was complete regression of the tumor and no others gave a favorable response, making a total of 2, or 4 per cent, in which the reaction was favorable. Of the corresponding 50 animals that received saline solution, none showed complete regression of the tumor and none revealed a favorable response.

PATHOLOGIC FINDINGS

Gross Examination

In control animals the tumors grew steadily and progressively until by the end of 3 weeks the average diameter was 41.34 mm. During the first 1½ weeks after transplantation the skin overlying the tumor was entirely normal but toward the end of the second week it exhibited "inflammation" (redness) in 53.27 per cent, scab formation in 10.27 per cent, and focal breakdown with discharge of necrotic tumor in 4.67 per cent of the animals (Table II).

TABLE II

Pathologic Changes in Skin Over Tumor

	Two weeks		Three weeks	
	Control	Hepbisul and natural thyroxin	Control	Hepbisul and natural thyroxin
Inflammation	53.27%	43.79%	10.93%	4.76%
Scab	10.27%	17.24%	56.25%	50.78%
Discharge	4.67%	8.04%	20.31%	19.04%

By the end of the third week the "inflammatory" reaction decreased to 10.93 per cent but scab formation soared to 56.25 per cent and focal breakdown with discharge increased to 20.31 per cent of the animals. Grossly, the tumors were always sharply circumscribed and did not invade the adjacent tissues. A definite capsule did not surround the mass but sometimes the neighboring connective tissue disclosed considerable edema. The tumors ordinarily were solid throughout until 10 to 12 days after transplantation, when they began to show central necrosis. As the breakdown of neoplastic tissue progressed it was sometimes accompanied by moderate degrees of liquefaction, but in none of the animals did the entire tumor liquefy (Figs. 1 and 2). The fluid was usually straw-colored and clear or opaque, but occasionally it was somewhat hemorrhagic. The peripheral portions of even the largest tumors showed grayish white, moderately firm, somewhat gelatinous, viable neoplastic tissue.

In animals treated with hepbisul and natural thyroxin there was definite retardation of growth in practically all of the tumors during the first 3 or 4 days. Following this initial depression the tumors (1) remained stationary in size or continued to lag far behind the controls throughout the experiment, or at the end of the third or fourth week (after transplantation) they either regressed completely or suddenly began to grow vigorously, (2) continued to regress and by the end of the week completely disappeared, or (3) began to grow but still lagged behind the controls (Figs. 1 and 2).

By the end of the second week (after transplantation) the average diameter of the tumors in control animals was 30.06 mm., whereas in animals treated with hepbisul and the natural thyroxin it was 25.20 mm. (Table III). By the end of the third week the average diameters were 41.34 mm. and 34.06 mm., respectively. These figures do not include the tumors that completely regressed. When they are incorporated, the average diameter of the growths in the treated animals dropped to 20.88 mm. at the end of the second week and to 25.55 mm. at the end of the third week. "Inflammation," scab formation, and focal breakdown with discharge of necrotic neoplastic tissue started sooner and

TABLE III

Average Diameters of Tumors

Treatment	One week	Two weeks	Three weeks
	mm.	mm.	mm.
Control	11.50	30.06	41.34
Hepbisul and natural thyroxin	11.82	25.20	34.06
Hepbisul and synthetic thyroxin	10.94	29.93	41.96
Hepbisul	11.44	28.42	39.26
Natural thyroxin	11.37	30.15	43.08

was of greater severity in treated than in control animals. By the end of the second week "inflammation" was already on the wane in treated animals (43.79 per cent), while in control animals it was reaching its peak (53.27 per cent) (Table II). Scab formation, however, in treated animals exceeded that in controls by over one-third, and discharge in treated animals exceeded that in controls by approximately one-half. By the end of the third week, control animals with an "inflammatory" response still outnumbered treated animals with a similar response, but scab formation and discharge were essentially the same in both groups.

The chain of events in animals treated with hepbisul and synthetic thyroxin, hepbisul alone, and natural thyroxin alone, was similar to that in control animals (Table III).

Microscopic Examination

In order to understand better the histologic changes induced by hep-

bisul and thyroxin, it seems desirable first to outline briefly the normal microscopic appearance of our particular sample of the Walker rat carcinoma 256 and to point out the changes which may occur as the result of post-mortem autolysis.

In *control animals*, the peripheral portions of the tumor in 21 day transplants and the entire tumor in 7 to 12 day transplants were composed of almost solid *viable neoplastic tissue*. The connective tissue stroma was so scanty that for practical purposes it was non-existent. Capillaries were not numerous and those present were inconspicuous. In the majority of tumors the cells were moderate to large in size. As a rule, the borders were indistinct and the cytoplasm of one cell merged with that of another to give diffuse syncytial sheets (Fig. 3). Less frequently the borders were more distinct and the cells then appeared polyhedral. As the outlines became sharper and the cells enlarged, their contours became distinctly rounded. In the smaller cells, the cytoplasm was moderate in amount, homogeneous, finely granular, and lightly eosinophilic (Fig. 4). In the larger cells it became more abundant and lighter staining, until definite small and later coalescing vacuoles appeared (Fig. 5). Some of these took the sudan stain and therefore represented accumulations of fat, while others remained clear and presumably represented hydropic degeneration. The nuclei were sharply defined, round, oval, or slightly irregular and light staining. The chromatin was coarse and clumped, and nucleoli were quite prominent. Mitotic figures were both regular and irregular and their number varied from 6 to 25 per high-power field. Occasionally, particularly in transplants up to 2 weeks of age, some of the tumor presented a glandular arrangement (Fig. 3). The glands were round or elongated, and one or several cell layers thick. The cell borders usually were indistinct and the cytoplasm formed syncytial masses. Occasionally the cells were more sharply delineated and the outlines were cuboidal. The cytoplasm was lightly eosinophilic and granular to reticulated but contained no vacuoles. The nuclei were similar to those encountered in cells presenting a diffuse non-glandular arrangement. Rarely the cells were long, spindle-shaped, and sarcoma-like (Fig. 6). They were fairly sharply demarcated and the cytoplasm was moderate in amount and densely eosinophilic. The nuclei were round, oval, elongated or irregular, and possessed a sharp delineating membrane and dense granular chromatin. At other times the nucleoplasm was more solid and distinctly hyperchromatic. Mitotic figures were less frequent than they were in the more rounded cells.

In control animals, killed 3 weeks after transplantation, the central portion of the tumor usually showed complete and diffuse, but some-

times partial and focal, areas of necrosis. *Complete necrosis* was manifested by an absolute disappearance of the normal architectural pattern and a replacement with an abundant amount of amorphous, pink-staining material. When the disintegration of cells was not quite complete, necrosis was represented by faint outlines of former neoplastic cells, nuclear fragments, and, occasionally, shadows of nuclei. In either case the vessels were inconspicuous. In the areas of more complete necrosis, calcification, in the form of bluish staining granular material or small spherical bodies, sometimes was present in the focal areas, but fibrosis was never seen. In tumors showing *focal necrosis* the dead tissue appeared similar. Interspersed between these foci, however, there were collars of viable or degenerating neoplastic cells surrounding engorged capillaries. The viable cells were similar to those seen in the more solid areas at the periphery, whereas the *degenerating cells* usually were distinct, rounded, separated from their neighbors, and smaller than is normal (Fig. 7). The cytoplasm was densely eosinophilic and moderate in amount. The nuclei were small, round, oval or irregular, and showed varying degrees of pyknosis, karyorrhexis, and karyolysis. Progression of these changes led to complete disintegration of the cells.

Sections of tumors from control animals that had died showed varying degrees of *post-mortem autolysis*. Frequently the capillaries were large, prominent, and engorged. Old foci of complete necrosis were similar to those already described. The tumor cells that were viable at the time of death were present in sheets at the periphery or were aggregated around vessels and disclosed varying, but in a given tumor uniform, degrees of change. They usually were shrunken, small, irregular, and might contain elongated cytoplasmic processes. The cytoplasm was moderate in amount, still somewhat granular, and either lightly eosinophilic or basophilic. The nuclei showed beginning condensation. They were round, oval, or irregular, deeply stained, and contained large blue granules. Mitotic figures were absent. With progression of these changes the cells became rounded off and smaller. The cytoplasm was moderate in amount and densely eosinophilic, and the nuclei became darker, smaller, and more pyknotic. Further progression disclosed liquefaction or coagulation necrosis. The former was manifest by disintegration and disappearance of some of the cells, greater shrinkage and pyknosis of others, and partial disintegration with fraying of the peripheral portions of the cytoplasm and karyorrhexis of the nuclei in still others. In coagulation necrosis the cell outlines were indistinctly discernible; the nuclei were completely, or almost completely, faded, and the chromatin granules from disintegrated nuclei were irregularly and diffusely scattered. Beyond this there was complete disin-

tegration of the cells, leaving a mass of amorphous, pink-staining débris speckled with fine, powdery, nuclear remains.

The pattern of changes observed in animals treated with *hepbisul and thyroxin* was distinctly different from that observed in saline-treated animals or from animals that had died and showed post-mortem autolysis. In order to obtain an understanding of the pathogenesis, some of the animals used in preliminary tests were killed at varying intervals from the 8th to the 21st day after transplantation. The picture on and beyond the 21st day was obtained from animals used in the experiment proper.

After two injections of hepbisul and thyroxin there were no foci of necrosis but the entire tumor nevertheless was affected. With low-power microscopic examination the uniformity seen in tumors from control animals was lacking and was replaced with irregular light and dark staining patches, streaks, and foci. The capillaries were inconspicuous. With higher magnification the most striking feature was a disruption of the cells. This occurred in ill defined focal areas that merged with adjacent neoplastic tissue. In less affected areas the cells showed a slight degree of over-all distortion (Fig. 8). They were, however, irregular, ill defined, smaller than usual, and contained scanty, homogeneous, or somewhat vacuolated cytoplasm. The nuclei were less prominent, as a rule more lightly stained, but some showed also varying degrees of pyknosis. In more severely affected areas the cells were fragmented, separated, and of irregular shapes and sizes (Figs. 9 and 10). The borders were broken and the cytoplasm was scanty and peripherally frayed. The nuclei were extremely irregular and pyknotic.

Following the initial disruption of the over-all pattern and cell structure, the destruction proceeded along one of two lines. On the one hand, necrosis, both liquefactive and coagulative, progressed unabated. The cells showed further distortion, disintegration, and liquefaction. Concomitantly, the products of disintegration were absorbed into the blood stream and the tumor gradually decreased in size and was ultimately completely absorbed. In such instances there was either no surrounding connective tissue reaction and the skin and subcutaneous tissue were returned to normal, or the resultant fibrosis might be minimal leaving only a small scar in the dermis (Fig. 11). On the other hand, the degeneration and disintegration of the tumor cells were accompanied by a rather marked fibroblastic proliferation. The fibroblasts originated throughout the tumor area from the inconspicuous supporting stroma and did not invade from the adjacent capsular region. More frequently the proliferating connective tissue formed branching and interlacing bands that separated and surrounded foci of tumor cells

(Figs. 12, 13, and 14). At first, the strands were composed of long, ill defined, spindle-shaped fibroblasts with a relatively large amount of light staining, somewhat granular, eosinophilic cytoplasm and oval, elongated or spindle-shaped, evenly and lightly stained, nuclei. Scattered throughout these areas there were varying numbers of neutrophils, eosinophils, and lymphocytes. Capillaries were inconspicuous. Later the leukocytic cells tended to disappear; the capillaries became even less prominent, and the fibroblasts became transformed into fibrocytes. The foci of tumor cells that seemed to be entrapped in the meshes of the proliferating tissue continued to exhibit progressive degrees of necrosis, liquefaction, and disintegration. Ultimately they showed complete disintegration, and were either resorbed and replaced by fibrous tissue, or they remained as masses of amorphous débris in which calcium salts were precipitated (Fig. 15). The latter existed as minute, fine granules or as larger, irregular clumps of intensely bluish staining material. About the periphery of the necrotic and calcified foci, giant cells of foreign body type sometimes became quite numerous. Throughout the connective tissue that had replaced most of the original tumor there also were frequently present numerous large, polyhedral or rounded, sharply defined foam cells containing fat droplets in their reticulated cytoplasm, and exhibiting small, round, somewhat eccentric, evenly stained, uniform appearing nuclei (Fig. 16). In addition, fatty material was sometimes crystallized in the form of long, spindle-shaped needles which, in sections stained with hematoxylin and eosin, were represented as empty spaces (Fig. 17). Leukocytic infiltration throughout the connective tissue was always minimal or absent.

Less frequently, fibrous tissue appeared to spring up diffusely, uniformly, and heavily throughout the tumor rather than in scattered branching strands (Fig. 18). In such cases the tumor cells were separated and existed singly or in clumps of two or three. They showed varying degrees of degeneration followed by necrosis and then complete disappearance. Upon their absorption the connective tissue simply condensed and ultimately existed as a solid mass of fibrous tissue.

Discussion

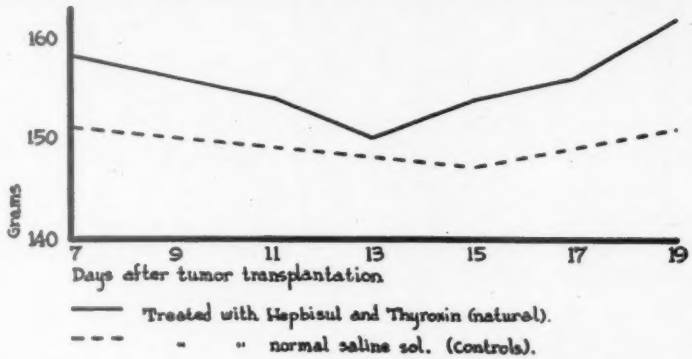
The *results* of this experiment clearly indicate that hepbisul combined with natural thyroxin is an effective inhibitor of the Walker rat carcinoma 256. Strong's statement in 1947,[16] that heptyl aldehyde of itself was not enough but that other chemicals in combination with heptyl aldehyde would produce a salutory effect upon the tumor, is thus borne out.

The histologic appearance of the tumor we used is similar to that

used by Earle,[17] who also obtained a sample from Columbia University. While the regressive changes that usually occur in the central portion of the tumor consist of degeneration, necrosis, liquefaction and absorption, and appear similar when due to post-mortem autolysis and when induced by hepbisul and thyroxin, it is a combination of factors which allows a distinct differentiation among the three processes. Spontaneous *ante-mortem changes*, for example, occur centrally. They are focal when the tumor is young but diffuse when it is older. In none of our animals, however (and we have examined several thousand already), are these spontaneous changes associated with fibroblastic proliferation. Also in the tumors that show spontaneous retrogressive changes the neoplastic tissue at the periphery is always vigorously viable. As a rule it consists of solid sheets of neoplastic cells with an extremely scanty or imperceptible stroma. Foci of necrosis, when they do occur, are likewise unaccompanied by fibrous tissue reaction. Occasionally, there is an increase of fibrous tissue about the circumference of the tumor, but this does not invade the neoplastic tissue and the two are readily separable. The *autolytic changes*, too, present a definite pattern and are easily distinguishable from the changes induced by hepbisul and thyroxin. The degeneration, necrosis, and liquefaction are similar, but they affect the entire mass, peripherally as well as centrally, and are not present in focal areas only. There are, therefore, no viable and vigorous tumor cells present and, of course, there is no fibrosis. The changes induced by ***hepbisul and natural thyroxin*** early in the process, to be sure, also consist of degeneration, necrosis, liquefaction, and absorption of neoplastic cells throughout the tumor, but, in addition, there is usually a diffuse increase of connective tissue that doubtlessly springs from the existing stroma. In our experience the odd tumors that regress spontaneously almost always show a complete resorption of the neoplastic tissue and complete restoration of the adjacent tissues to normal. While this occurs also in animals that have been treated with hepbisul and thyroxin, more often the tumor cells, instead of being completely liquified and resorbed, become calcified and the calcific foci are surrounded by foreign body giant cells and fibroblasts. In some only the fibrous tissue remains.

Although it is well known that an inadequate or starvation diet will result in retrogressive changes in tumors, we believe that inadequacy of food intake played no part in our experiment. Both the control and the treated animals were kept under similar experimental conditions, were fed the same diet, consumed approximately similar portions, and maintained essentially parallel weights (Text-Figs. 1 and 2). Animals in both groups upheld their weights until approximately the 21st day, at

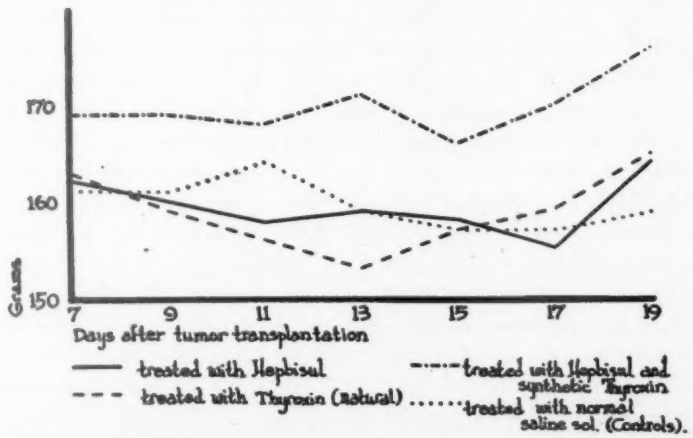
which time, if the tumors were large and showed considerable central necrosis and liquefaction, they began to lose weight. In other words the loss in weight was terminal, was parallel in both control and treated animals, and probably resulted from absorption of toxic material from the tumor rather than from a decrease in consumption of food. In addition, none of the large tumors in either group revealed more than cen-



Text-Figure 1. Curves presenting the average weights of 100 tumor-bearing rats of each of the two groups indicated.

tral regressive changes histologically. Fibrosis was not present. As far as we could determine, infection likewise played no part in either the control or the treated animals.

At present we have no facts regarding the mechanism of action. Since the retrogressive changes in the tumor cells are far in advance of



Text-Figure 2. Curves presenting the average weights of 50 tumor-bearing rats of each of the four groups indicated.

the connective tissue proliferation, there seems to be no doubt that the drugs act upon and destroy the tumor cells directly rather than by a roundabout process of fibrosis. We have no explanation for the fact that natural thyroxin in combination with hepbisul is effective while synthetic thyroxin is not. With the doses employed, neither product resulted in a clinically visible metabolic change in any of the animals. Because there is this distinct difference in the response induced by the natural and synthetic products, the first assumption is that thyroxin acts not by increasing the metabolism and multiplication of the tumor cells but by some other synergistic means. From a chemical point of view, however, there is another possible explanation which we have not as yet pursued. Natural thyroxin is levorotatory, while synthetic thyroxin consists of equal parts of both the levorotatory and dextrorotatory fractions. Since the levorotatory fraction alone is responsible for the increase in metabolism in the body, it would appear that to obtain the desired effect the dose of synthetic thyroxin should be twice that of the natural product. The amount of natural and synthetic thyroxin used in our experiment, however, was the same.

Statements in the literature regarding the toxicity of heptyl aldehyde sodium bisulfite are not in agreement. Strong [16] was of the opinion that the preparation was relatively innocuous, whereas Garai [15] stated that the compound was not a suitable chemotherapeutic agent for cancer because its destructive effects on the kidneys and liver far outweighed any beneficial action. Our experience supports the observations made by Strong. When the amount of hepbisul used is small it may be injected subcutaneously, intraperitoneally, or intravenously with impunity. When the amount used is relatively large (as in our experiment), repeated subcutaneous injections in the same area will produce local irritation and moderate necrosis in approximately one-half the animals. Histologic examination of portions of the liver and kidneys from both tumor-bearing and normal animals, given similar amounts of hepbisul, revealed no untoward histologic changes. Some of the kidneys showed slight tubular degeneration, but at no time was necrosis or other damage encountered. Furthermore, Gruber has just completed full toxicity studies on hepbisul (to be published separately) and states that the compound is quite innocuous. He reports that the LD 50 in rabbits is 0.45 gm. intravenously per kg. of body weight and in rats 1.3 gm. intraperitoneally per kg. of body weight. Histologic examination of the livers and kidneys of some of the animals used by Dr. Gruber, that received large amounts of hepbisul, likewise revealed no pathologic changes.

Summary

Hepbisul and natural thyroxin were administered subcutaneously to Sprague-Dawley rats bearing the Walker rat carcinoma 256. Of the 108 animals treated, 27 showed complete regression of the tumors and 12 others showed a favorable histologic response. A salutary effect, therefore, occurred in 39 animals or 36 per cent of the total.

The retrogressive changes consisted of degeneration, necrosis, liquefaction, and fibrosis. The chemicals apparently act directly upon the tumor cells and the fibrosis is compensatory and secondary.

Hepbisul and synthetic thyroxin resulted in a favorable response in 2 of 50 animals treated or a total of 4 per cent. The cause of this discrepancy is unknown.

Despite the relatively high doses used, local irritation and ulceration at the point of injection occurred in less than 50 per cent of the animals, while general toxic effects, as manifested by morphologic changes in the liver and kidneys, were not encountered.

It is concluded that hepbisul and natural thyroxin when administered subcutaneously are effective inhibitory agents for the Walker rat carcinoma 256 in rats of the Sprague-Dawley strain.

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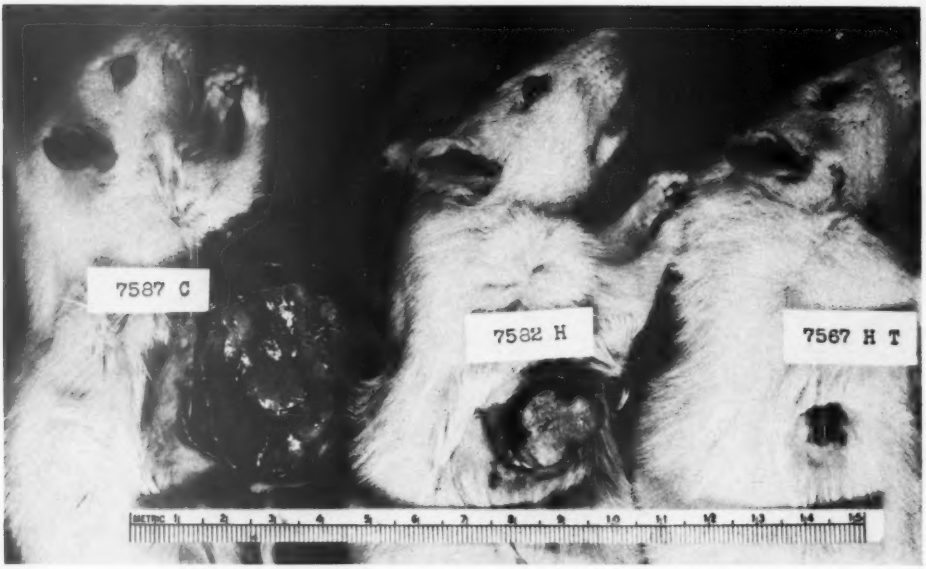
DESCRIPTION OF PLATES

PLATE 12

FIG. 1. Gross appearance of three hemisected tumors *in situ*. The growth in the control animal (C) is large and shows central necrosis and liquefaction; that in the animal treated with hepbisul alone (H) is small and shows central necrosis but no liquefaction, while the tumor in the animal treated with hepbisul and natural thyroxin (HT) has completely disappeared.

FIG. 2. Cross sections of two tumors from control (C) animals both male (M) and female (F) showing central necrosis without liquefaction. The remaining specimens (T) are from treated male (M) and female (F) animals in which the tumors had entirely disappeared or were replaced by yellowish foci of complete necrosis surrounded by fibrous tissue.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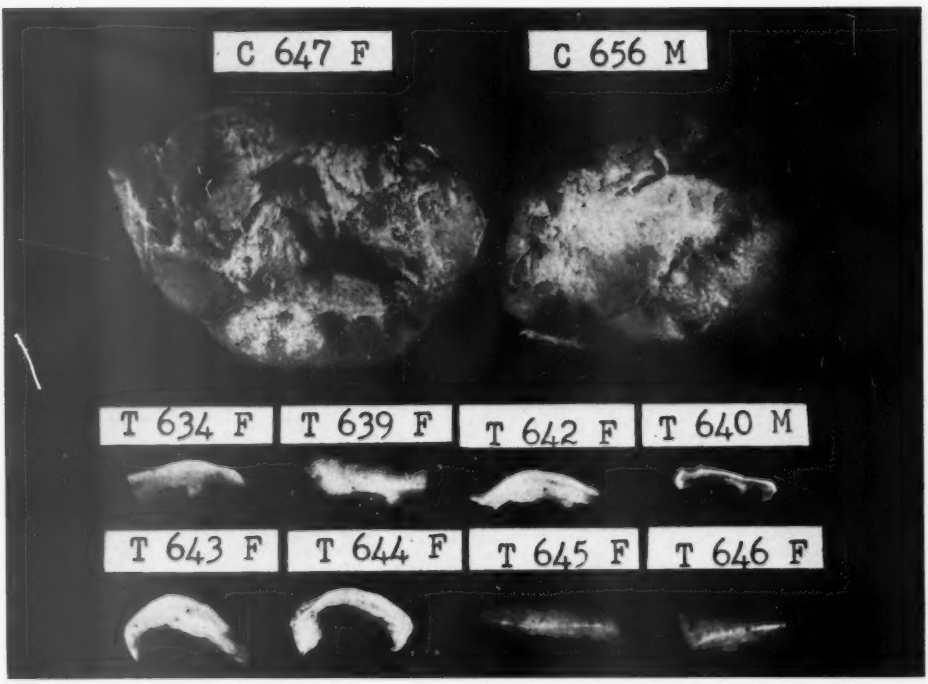
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T 639 F
T 642 F
T 640 M
T 643 F
T 644 F
T 645 F
T 646 F

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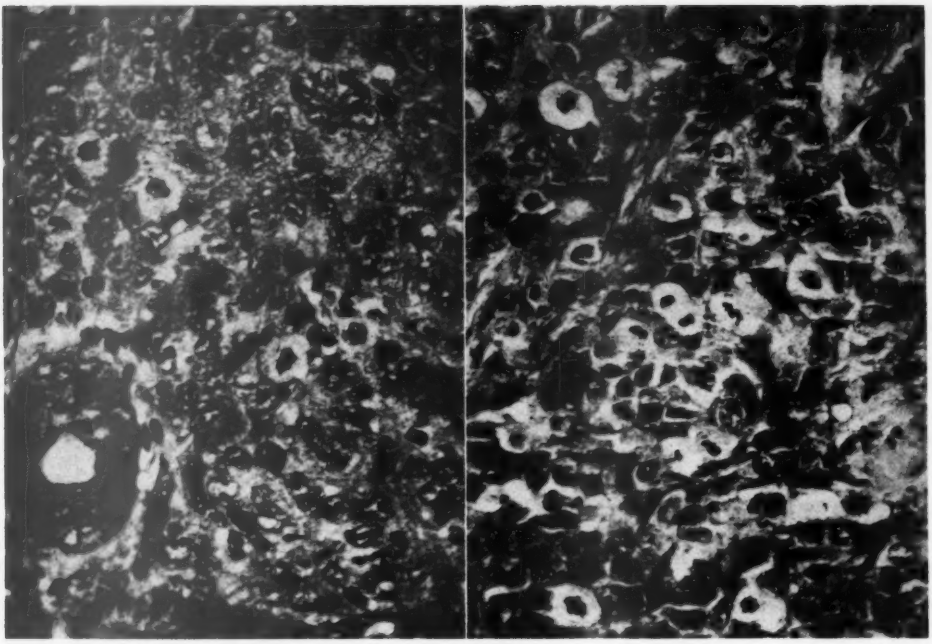
PLATE 13

FIG. 3. Tumor from control animal showing diffuse sheets of large, ill defined cells and one well formed gland. × 380.

FIG. 4. Tumor from control animal showing more distinct, rounded or polyhedral cells, some of which contain light-staining cytoplasm. Mitotic figures are numerous. × 380.

FIG. 5. Tumor from control animal showing large, round, often vacuolated neoplastic cells. × 380.

FIG. 6. Tumor from control animal exhibiting sarcoma-like cells. × 380.



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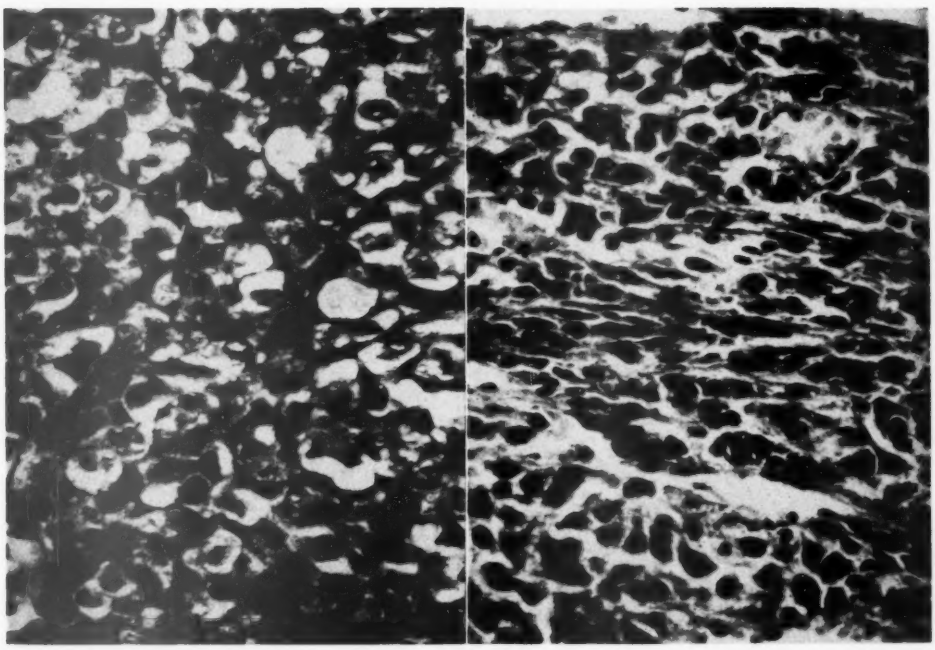

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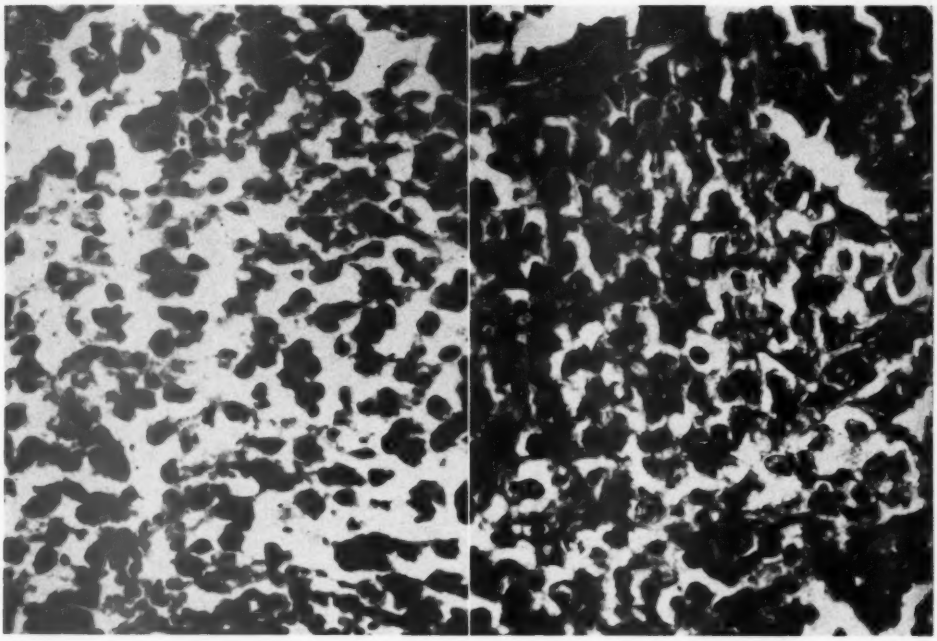
PLATE 14

FIG. 7. Tumor from control animal illustrating spontaneous degeneration and liquefaction. × 375.

FIG. 8. Tumor from animal that received one injection of hepbisul and natural thyroxin, showing an over-all disruption of architecture and beginning disintegration of the neoplastic cells. × 375.

FIG. 9. Tumor from treated animal illustrating progression of the degenerative changes seen in Figure 8. × 375.

FIG. 10. Tumor showing further progression of the changes illustrated in Figures 8 and 9. Pyknosis, karyorrhexis, and liquefaction are apparent. × 375.



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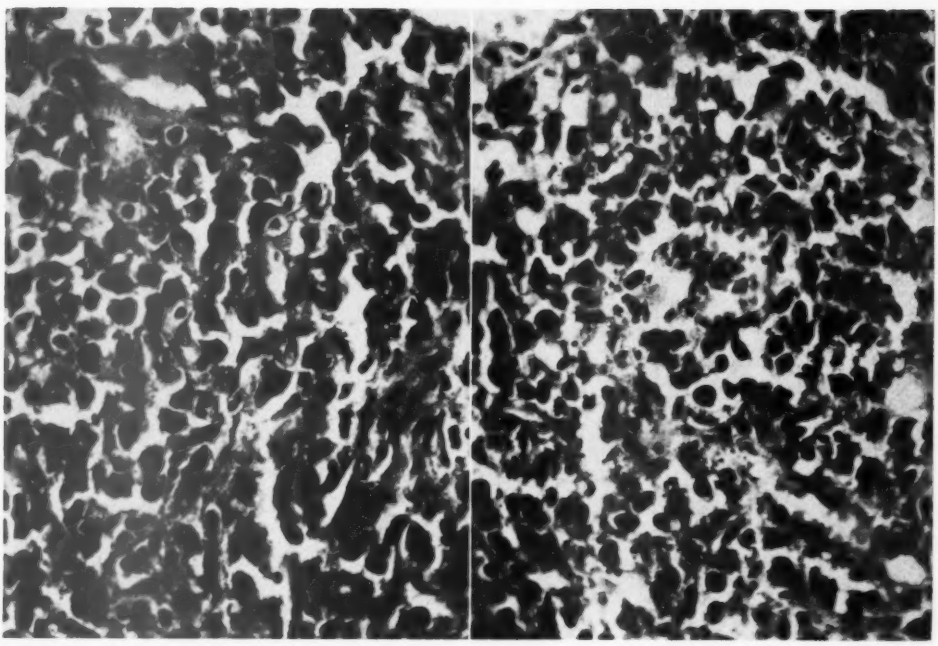

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PLATE 15

FIG. 11. Tumor site from treated animal showing only a scar in the dermis. The tumor has completely disappeared. × 95.

FIGS. 12, 13, and 14. Tumors from treated animals showing varying degrees of fibrosis and of necrosis and liquefaction of neoplastic cells. × 375.

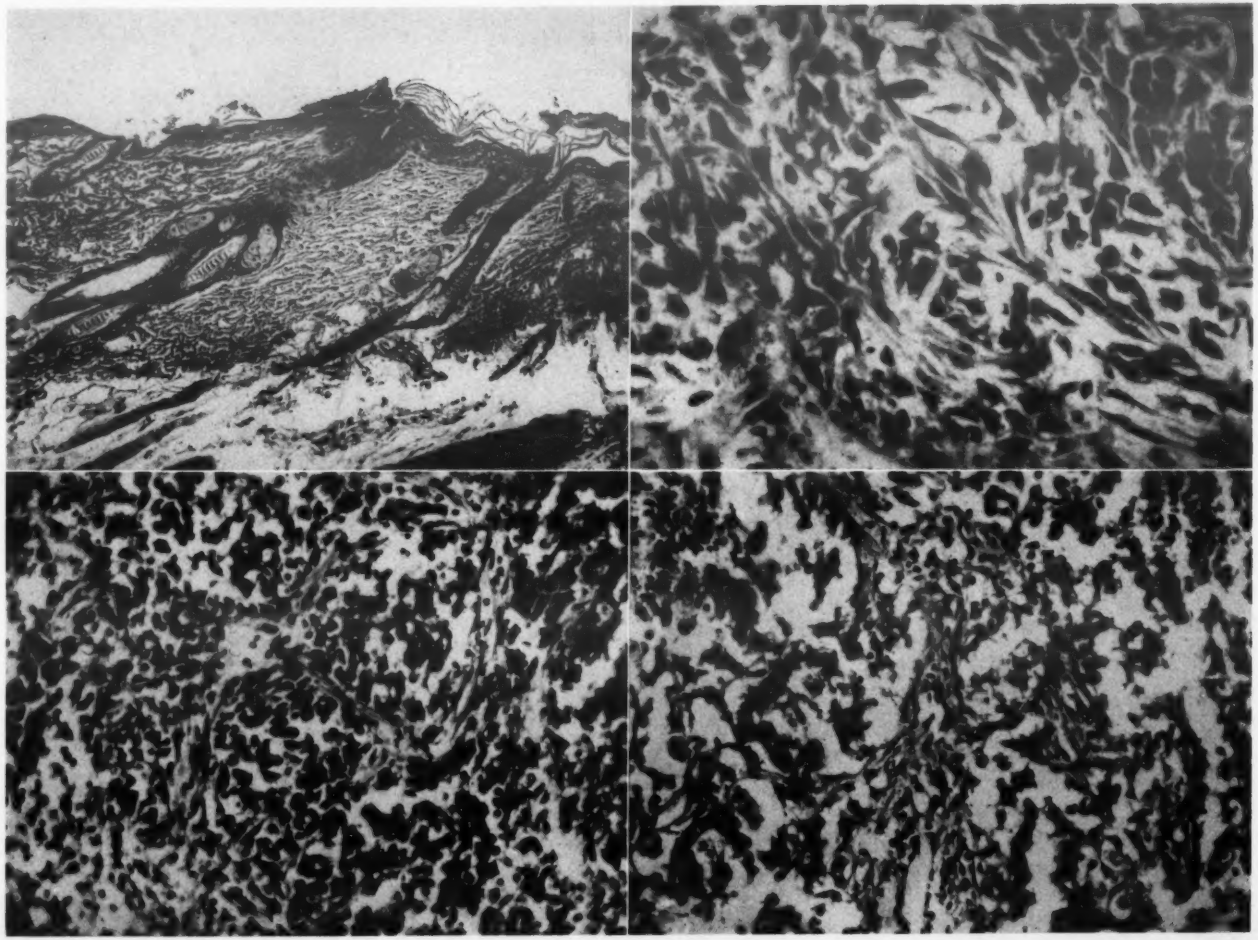

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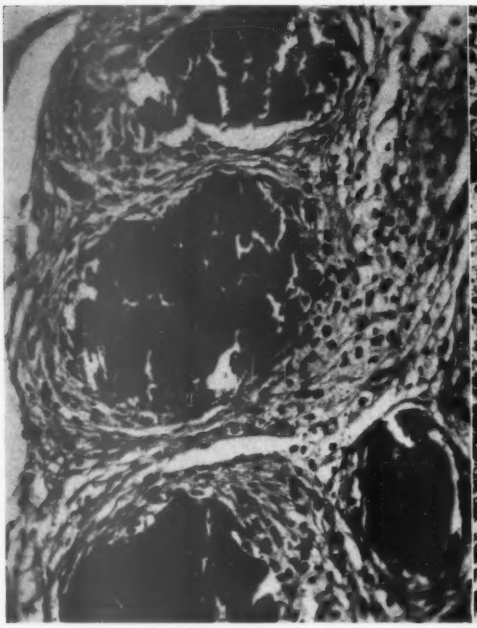
PLATE 16

FIG. 15. Tumor site from treated animal showing fibrous tissue, foam cells, and giant cells surrounding calcific foci. × 185.

FIG. 16. Fatty material deposited at the site of a previous tumor in a treated animal. Sudan II stain. × 95.

FIG. 17. Tumor site from treated animal showing only foam cells and crystals. × 185.

FIG. 18. Tumor from treated animal illustrating a diffuse increase of fibrous tissue enmeshing single neoplastic cells in various stages of necrosis. × 375.



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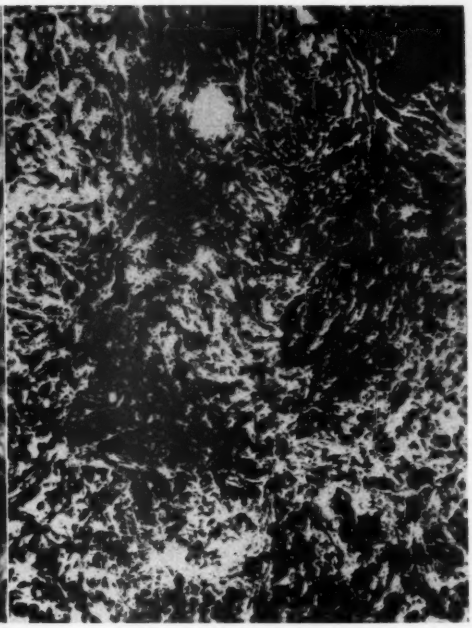

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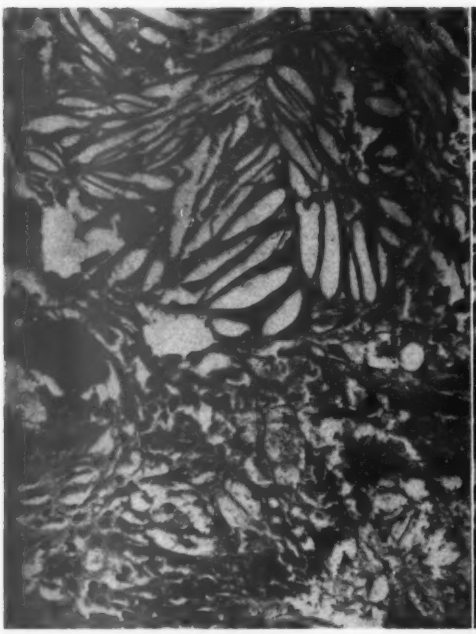

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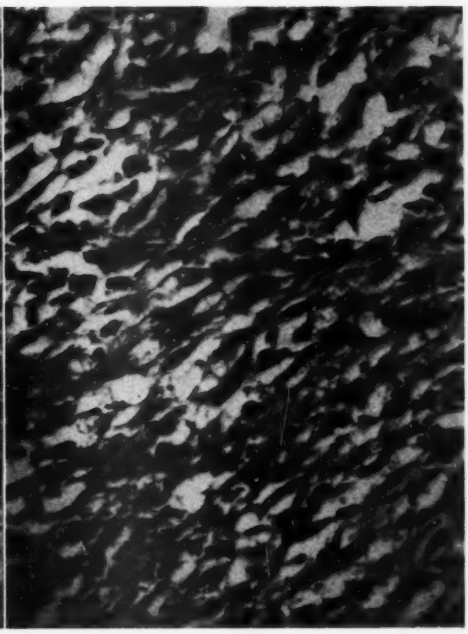

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Hepbisul and Thyroxin on Carcinoma 256

CONGENITAL GOITER IN NORTH AMERICA *

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Congenital enlargement of the thyroid gland in North America is a very unusual occurrence. Not only is the number of reported cases small, but the number of cases with pathologic study is only 11.

Congenital enlargement of the thyroid gland was first described by Fodéré [1] in 1796 and since that time well over 400 cases have been documented. Of this group, the great majority were clinical studies in continental Europe. Crotti [2] cited 37 congenital goiters of 642 goiters reported by Demme,[3] 25 of 2,292 by Diethelm,[4] and 42 of 1,996 by Richard.[5] In striking contrast to this there have been no reports of cases from the goiter clinics of this country and, in spite of a high incidence of juvenile and adult goiters, congenital goiter has remained a rare, sporadic occurrence even in the American goiter belt. Perhaps the highest incidence of this condition is in the State of Washington where Skinner [6,7] has reported 19 clinical cases and one which was studied post mortem.

The most obvious feature in the genesis of congenital goiter is the presence of goiter in the mother. In European reports between 50 and 100 per cent of the mothers had goiters and a somewhat similar situation has been found in this country. Iodine deficiency has been incriminated as the most important cause of congenital goiter. This has been empirically demonstrated by the great prophylactic value of prenatal iodine therapy.[7] Congenital goiter has been reported to occur when mothers received excessive prenatal iodine.[8] Hyperplasia has been found in the thyroid glands of fetuses whose mothers were under thiouracil therapy.[9,10] This indicates that goiterogenic drugs such as sulfonamides, thiouracil derivatives, and thiocyanates might be responsible for congenital goiter. Some cases have obscure etiologic factors and fall into an idiopathic category.

The course of congenital goiter in those who survive the early postnatal period is usually that of a regression of the mass, probably accelerated by iodine therapy.[2] The major problem of congenital goiters when they are large is the pressure on the larynx and trachea, especially when the lateral lobes encroach on the posterior surface. This so-called circular goiter severely interferes with the air passageway, and asphyxia is the chief cause of death. Large, encircling, parenchymatous goiters

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are usually fatal. The goiters of vascular origin usually afford a good prognosis.

The pathologic anatomy of congenital goiter is essentially that of an intense, diffuse hyperplasia and hypertrophy of the thyroid gland. The normal range of weight for the thyroid gland of a newborn infant is between 1.5 and 2.5 gm. A thyroid gland such as seen in case 1, summarized below, represents at least a twenty-fold increase in size. The hypertrophy is usually symmetric, although asymmetry or substernal extensions may occur.

As to microscopic structure, diffuse parenchymatous, telangiectatic, fibrous, colloid, cystic, and adenomatous varieties have been described. The diffuse parenchymatous type is essentially due to follicular hyperplasia and is the most commonly encountered variety. There is diffuse cellular hyperplasia, expressed as follicular hyperplasia with high-cuboidal or columnar epithelium lining acini with little or no colloid; or there may be follicles filled with loose, apparently desquamated cells. A telangiectatic or vascular variety has been described frequently in the older foreign literature. Two types have been found [11]: In smaller glands, a massive hyperemia, probably related to labor, has produced increased size; in larger glands weighing from 17 to 36 gm., the majority of the increased weight is assigned to congested, hyperplastic, and hypertrophied blood vessels, particularly perifollicular capillaries. Hemorrhage within these glands may occur. Colloid goiters and fibrous goiters have been reported rarely. Cystic goiters containing cysts filled with brown liquid, rich in cholesterin, lined by epithelium, and having cartilage in the walls, have been described. Crotti [2] believed that most of the larger of these cystic goiters are teratomas. Rarely adenomatous or nodular congenital struma has been described.

In the North American medical literature 47 cases of congenital goiter have been reported, of which the great majority of reports concern clinically diagnosed cases which recovered with or without therapy. Five cases [12-16] were treated surgically. These were operated upon from the 12th day to the 18th month, either because of pressure symptoms or failure to regress (Table I). Two of the glands apparently were parenchymatous goiters, one a colloid goiter, and 2 were designated as Hürthle cell tumors. Six fatal cases [7,12,17-20] with autopsy findings have been recorded in the American literature (Table II). In the 5 cases having microscopic descriptions, epithelial hyperplasia was described uniformly.

Symmers [13] has reported a case which he designated as a congenital Hürthle cell tumor. Morrow [14] later, apparently on the basis of Symmers' case, recorded another similar case. Whether or not these cases

differ substantially from the many other cases of congenital goiter does not detract from the fact that both were present at birth and thus fall into the category of congenital goiters. It would appear that they represent, at the most, variants of the usual parenchymatous congenital goiter.

TABLE I

North American Congenital Goiter Treated Surgically

	Maternal goiter	Age at time of operation	Thyroid gland removed	Microscopic appearance
Williamson[15]	Not stated	18 mos.	25.5 gm.	Diffuse hyperplasia
Peterson and Sondern[16]	Not stated	5 wks.	6 x 4 x 3 cm. and 4 x 2.5 x 2 cm.	Colloid goiter
Davies[13]	Moderate goiter	12 days	6 gm.	Subinvolution of hyperplastic gland
Symmers[13]	Not stated	6 wks.	47 gm.	Hürthle cell tumor
Morrow[14]	Not stated	2 mos.	4 x 2 x 1.5 cm.	Hürthle cell tumor

As the number of recorded autopsied cases of congenital goiter is only 6, and no adequate presentation of the pathologic anatomy has appeared in the American literature, the following 2 cases of fatal congenital goiter with autopsy findings are presented.

TABLE II

North American Cases of Congenital Goiter with Necropsies

	Maternal goiter	Survival period	Size of thyroid gland	Microscopic appearance
Skinner[7]	None	7.5 hrs.	16.3 gm.	Not given
Abt[17]	Not stated	Died soon after birth	Size of walnut	Vascular and hyperplastic
Mitchell and Struthers[18]	Not stated	6 wks.	12 cm. in circumference at isthmus	Fibrosis and hyperplasia
Hill[19]	Goiter	4 days	57 gm.	Hyperplasia with considerable colloid
Solis-Cohen and Steinbach[20]	Myxedematous appearance	48 hrs.	41 gm.	Hyperplasia
Davies[13]	Medium goiter	13 days	Each lobe 4 cm. in diameter	Marked hyperplasia
Case 1	None	½ hr.	50 gm.	Marked hyperplasia
Case 2	None	5 hrs.	20 gm.	Marked hyperplasia

REPORT OF CASES

Case 1

Baby boy C. The mother was a 24-year-old primipara. She received only ferrous sulfate and calcium as medication during her pregnancy. Hydramnios developed in the last trimester. Due to some cephalopelvic disproportion, delivery was somewhat difficult. The mother had had two subsequent pregnancies which were uneventful. At no time had she shown thyroid enlargement or symptoms.

The newborn infant never breathed properly and expired ½ hour after delivery. An autopsy was performed 18 hours after death. Body weight was 3890 gm.; length, 52 cm. External examination revealed slight overlapping of the parietal bones and

a large, soft mass in the anterior portion of the neck extending symmetrically from the clavicle to either side of the mandible. On internal examination the viscera other than the thyroid gland were within normal limits except for atelectatic lungs. The thyroid gland weighed 50 gm. and both lobes and the isthmus were symmetrically enlarged (Fig. 1). The surface was dark red-brown and the capsule was somewhat thickened.

Microscopically, the thyroid gland was composed of loosely arranged balls of cells with poorly preserved acinar linings which gave a desquamated appearance. The cells had a faintly acidophilic cytoplasm which usually was indefinite in outline. Most nuclei were of intermediate size but small and large hyperchromatic nuclei were common (Fig. 2). In the periphery of lobules the acini were small and had discrete, sharply outlined cells (Fig. 3). Colloid was absent throughout. A very prominent perifollicular distribution of hyperemic capillaries was present. Aside from a fairly thick collagenous capsule, connective tissue was not prominent.

Case 2

Baby boy A. The mother was a 29-year-old primipara who gave birth to the child at 6½ fetal months. Hydramnios was present which enlarged the uterus to the size of a term pregnancy. The baby was born by vertex presentation, although both arms were prolapsed. The child breathed poorly and expired 5 hours after birth. The mother showed no evidence of goiter and received no unusual medication.

The autopsy was performed 3 hours post mortem. External examination revealed a symmetric mass, 3 by 5 cm., in the anterior portion of the neck. On internal examination the thyroid gland was symmetrically enlarged, weighing 20 gm. Each lobe measured 4 by 2 by 1 cm. and anteriorly a nodule measuring 0.8 cm. in diameter was present on each side (Fig. 4). The gland surrounded the trachea but no definite compression was evident. The heart weighed 13 gm., and the left ventricle was somewhat compressed transversely by a narrow band of pericardial thickening. The other viscera were within normal limits for weight and appearance except for atelectatic lungs and a tentorial tear of 1 cm.

Microscopically, the thyroid gland was composed of follicles lined by high-cuboidal to columnar epithelium (Fig. 5). The cytoplasm was faintly acidophilic and the nuclei were round, uniform, fairly large, and hyperchromatic. Papillary infolding was present. A considerable number of follicles contained pink colloid. Scalloping of the edges of the colloid was not seen. Hyperemic perifollicular capillaries were present and connective tissue was minimal. Marked extramedullary hematopoiesis was present in the liver. A minute focus of polymorphonuclear cells was present in the pancreas. The endocrine organs and other viscera showed no unusual features.

Discussion

Anatomically, the congenital goiter does not differ greatly from the adult goiter. Fundamentally, the same cell types and arrangements are present, although marked vascularity and deficiency of colloid are more characteristic of the fetal gland. The microscopic appearance of the gland in most cases is that of a hypersecreting organ, but there has been no clinical evidence that hyperthyroidism is present. Symptoms and signs of reported cases are limited to those caused by the enlargement of the thyroid gland *per se*.

The thyroid glands in cases 1 and 2 each presented parenchymatous hyperplasia. The gland in case 1 was described as having a desquamated appearance. This feature of certain congenital goiters has bothered pathologists for over 50 years. Staemmler [21] believed that this is an intravital regressive change, in that the number of cells in a follicle are far too numerous to arise simply from desquamation of the lining epithelium, and in addition in a series of 24 fetuses he failed to find a relationship between the delay of autopsy after death and the degree of "desquamation." Unfortunately, since case 1 was autopsied 18 hours post mortem, it does little to clarify the issue even though other organs failed to show significant post-mortem changes.

Cretinism of the endemic variety is seen in the same regions as is congenital goiter and in European reports the two are commonly associated. This has suggested the thesis that prenatal iodine deficiency in the mother is the basis of each condition. Sporadic cretinism, on the other hand, is much less often associated with congenital thyroid enlargement, and athyreosis or aplasia is thought to be the usual basis for this variety of cretinism.

Summary

In two autopsied cases of congenital goiter the thyroid glands showed parenchymatous hyperplasia. The cause of most of these goiters is obscure although a disturbance in iodine metabolism appears to be the most prominent factor.

Grateful acknowledgment is due Miss Stella Zimmer and Mr. Louis Georgianna for the illustrative material.

Since submitting this manuscript, an additional autopsied case of congenital goiter has been reported (Seligman, B., and Pescovitz, H. Suffocative goiter in newborn infant. *New York State J. Med.*, 1950, 50, 1845–1847.) This case is of particular interest as the mother received propylthiouracil during her pregnancy for the control of hyperthyroidism. The newborn infant had a goiter weighing 16 gm., which caused asphyxia.

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DESCRIPTION OF PLATES

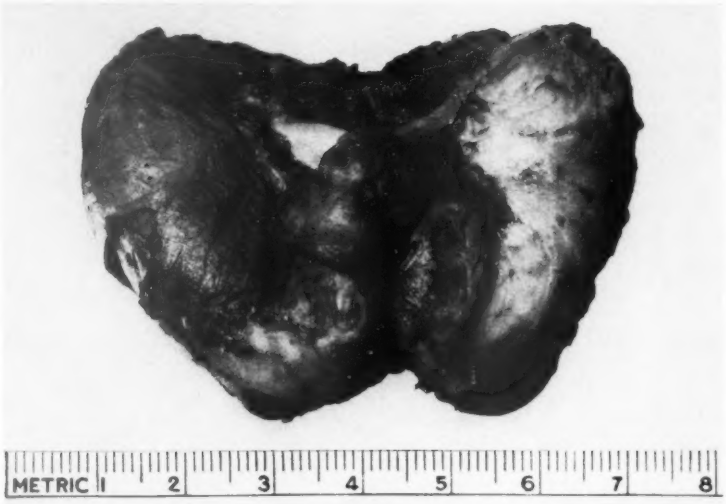
PLATE 17

FIG. 1. Case 1. Anterior view of thyroid gland.

FIG. 2. Case 1. Histologic structure of thyroid gland. Hematoxylin and eosin stain. × 565.

FIG. 3. Case 1. Subcapsular region of the thyroid gland. Hematoxylin and eosin stain. × 135.

1



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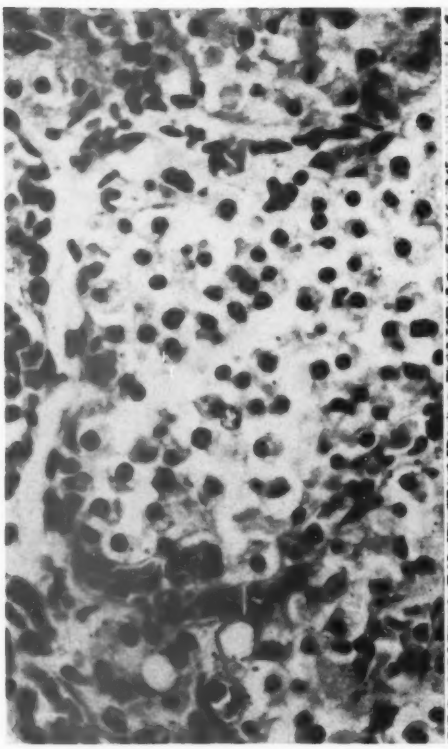

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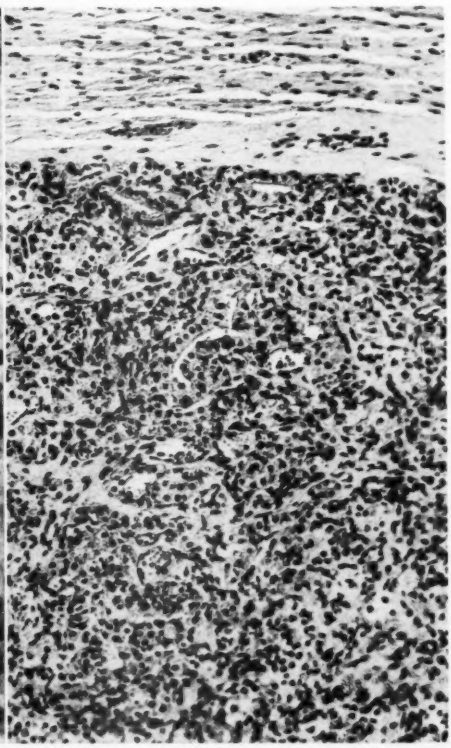
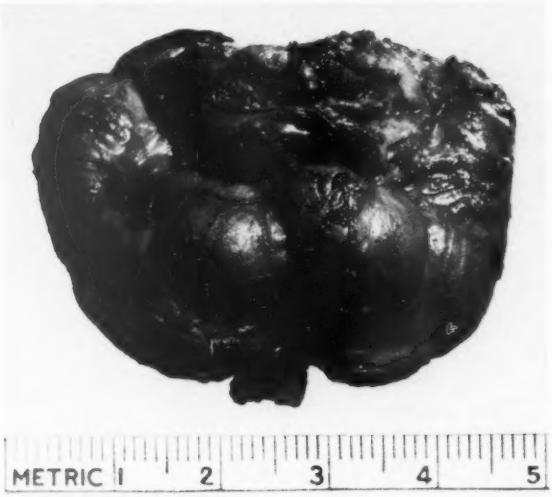


PLATE 18

FIG. 4. Case 2. Anterior view of thyroid gland.

FIG. 5. Case 2. Histologic structure of thyroid gland. Hematoxylin and eosin stain. × 135.

4



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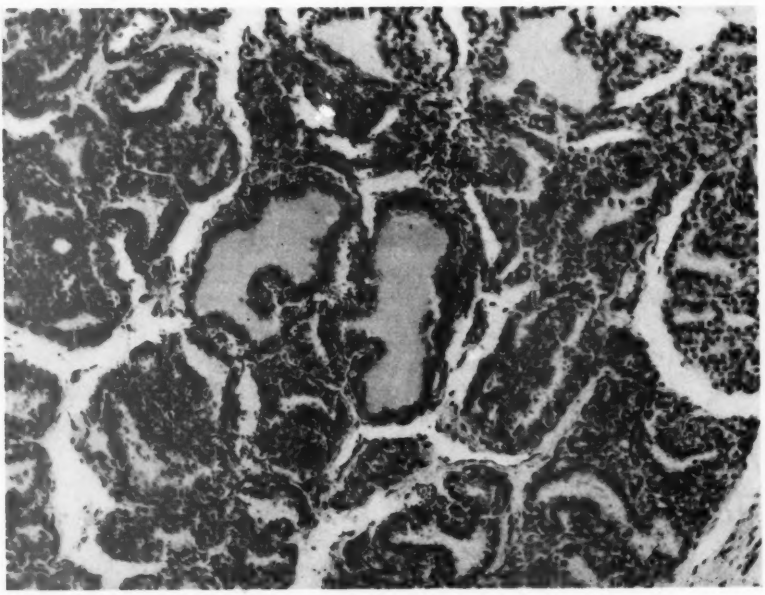

THE RENAL ARTERIAL VASCULATURE IN MAN *

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Two lines of recent experimental and clinical investigation have contributed to re-awakening of interest in the intimate arrangement of the renal vasculature in man. For some years past there has been considerable interest in the investigation of the renal circulation in relation to essential hypertension [1-5] and more recently Smith,[6] Van Slyke *et al.*,[7] Trueta *et al.*,[8,9] and others [10,11] have demonstrated a remarkable variation in the renal blood flow and in its route through the kidney under a variety of abnormal conditions. It is obvious that precise anatomical information as to the distribution and character of the blood vessels down to their finest ramifications is essential to a complete comprehension of the significance of such physiologic observations. The present study was undertaken with a view to determining whether the use of neoprene as an injection medium would provide the means of gaining information regarding certain essential details of the renal arterial vasculature in addition to that already available in the literature.

Huschke [12] and Bowman,[13] more than 100 years ago, were the first to liken the renal circulation to a portal system and to point out that all of the blood entering the parenchyma of the kidney passed first through the glomeruli before reaching the peritubular capillary plexuses. This view has been supported by the majority of subsequent investigators of the renal circulation, including Huber,[14] Lee-Brown,[15] MacCallum,[16] and others,[17-19] and is now generally accepted as the normal design of renal blood flow. However, this concept has been challenged in the past by Ludwig,[20] Virchow,[21] and others,[22] who were convinced that a significant number of arterial twigs, instead of ending in glomerular capillaries, passed directly into the intertubular capillary plexus. It is true that such direct channels are encountered in arterially injected specimens but they are few in number and it is generally agreed that they represent an acquired abnormality brought about, as MacCallum [23] has demonstrated, by progressive obliteration of the glomerular capillary tuft until the former afferent and efferent arterioles are brought into direct continuity. An even more radical departure from the basic plan of the renal circulation has been suggested by Steinach,[24] Golubew,[25] and Spanner [26,27] who have brought forward evidence interpreted by them as indicating the existence of arteriovenous anastomoses between small blood

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vessels within the kidney substance. Investigation of this possibility by Springorum [28] and Trueta *et al.*[9] led them to the conclusion that such arteriovenous communications, if they occur at all, must be extremely uncommon.

Great interest attaches to the recent publication of Trueta, Barclay, Daniel, Franklin, and Prichard [9] who have demonstrated in physiologic experiments that the blood flow of the outer two-thirds of the renal cortex may be re-routed through the juxtamedullary glomeruli and medulla. Suggestive evidence led them to the conclusion that the vessels of this portion of the kidney may carry a quantity of blood equal to that passing normally through the whole mass of cortical tissue, but no direct measurements of blood flow were made to support this statement. These authors were of the opinion that the anatomical pathway of this alternative route is principally by way of more or less direct vessels joining the arteriolae rectae of the medulla to the renal veins without the interposition of a medullary capillary bed.

It is clearly important to estimate the frequency of the direct vessels of the older literature (arteriolae rectae verae, Ludwig's arterioles, etc.) and of the types to which Trueta *et al.*[9] have recently drawn attention. Obvious value would also attach to precise measurements of the various components of the renal arterial vasculature from which could be calculated the total capacity of the renal arterial bed. Accordingly, a study of the renal arterial vasculature was undertaken employing neoprene casts of the renal arterial tree as described in a previous publication.[29]

Methods and Material

The kidneys to be studied were removed from human subjects as soon as possible after death; those removed less than 5 hours after death gave the best results. Care was taken to remove the kidney with as much of its surrounding adipose tissue as possible, and with the full length of renal artery and vein. A large canula was tied into the renal artery and connected with a cold water tap, water pressure being regulated by means of a mercury manometer. Perfusion was carried out at a pressure of 75 mm. of Hg for the first half hour, and at 150 mm. of Hg thereafter, until the washings coming from the renal vein were clear. Leaking was controlled by ligation of individual vessels and mass ligation of perirenal fat. The washed kidney was kept at 4° C. for 6 to 12 hours to allow for the escape of as much of the perfusion fluid as possible, and then removed to room temperature for about 5 hours to decrease edema and render the kidney pliable. The neoprene was forced from a closed vessel at a constant pressure of 150 mm. of Hg, maintained by air passing through a circuit containing a manometric escape valve. It

was found important to have the tubing completely filled with neoprene to the level of the renal artery and the pressure up to 150 mm. of Hg before the clamp was released to allow the entry of neoprene into the renal artery. Injection was continued usually for 3½ minutes. Artery and vein were then tied before the pressure was released.

At this point a few kidneys were fixed in 4 per cent formalin and 4 per cent acetic acid, and histologic sections prepared according to the method of Lieb.[30] However, after injection, most of the kidneys were corroded by placing them immediately in commercial hydrochloric acid at 56° C. The specimens were gently agitated from time to time and maceration was complete at the end of 24 to 36 hours, at which time the cast was cleaned by washing gently in warm water.

The larger vessels were studied by floating the whole cast, or portions of it, in water. For the observation, measurement, and photography of finer structures, small sprigs of vessels cut from the cast were mounted in Farrant's solution under a coverslip according to the technic previously described by us.[29] These mounts were studied under the microscope. The best photomicrographs were obtained by reflected light.

A total of 36 normal human kidneys were studied by this method. Twenty-five of these were from males. Among the 36 cases, each decade from the first to the eighth was represented. In all of these cases there were no urinary abnormalities during life indicative of disease of the kidneys. There was no history of hypertension and there was no hypertrophy of the heart at autopsy. In each case the opposite kidney presented a normal gross and microscopic appearance.

Observations

The typical appearance of a neoprene cast of a normal human kidney injected through the renal artery is shown in Figure 1. Examination of such a cast shows the main renal artery dividing into an anterior and a posterior group of arteries. The larger anterior group supplies about two-thirds of the kidney as pointed out by Brödel[31] (Figs. 1 and 2). In neoprene casts of the renal vessels, the anterior and posterior groups and their further ramifications readily fall apart, denoting the independence of these two groups of vessels and complete lack of anastomoses (Fig. 2). The only small vessels arising from these major divisions are a few minute arteries that supply the pelvis and peripelvic tissues within the hilus of the kidney.

To describe accurately the further divisions of the renal artery, it is necessary to describe the shape of the renal pyramids and the relationship of the pyramids to one another, to the cortex, and to the vessels. Each pyramid is like the head of a mushroom with only a small portion

of its stalk, the latter corresponding to the renal papilla. They form an anterior and a posterior row with their convex surfaces facing the anterior and posterior surfaces of the kidney, respectively, while the apices of the pyramids (the renal papillae) face one another across the pelvis of the kidney. The relationship of the major divisions of the renal artery to the pyramids and the cortical tissue which covers the convex surfaces of the pyramids is shown in Figure 3.

The 8 to 12 interlobar arteries are arranged to lie between adjacent pyramids so that each supplies the cortex covering the adjacent surfaces of two adjacent pyramids. The interlobar arteries divide into further branches which gain the corticomedullary interval at various points about the margins of the pyramids. These branches, the so-called arcuate arteries, divide by simple dichotomous division and their divisions fan out and course in various directions to form a vascular cap over the convex surface of the pyramid, from which is derived the whole arterial blood supply of the cortical tissue that lies over it (Figs. 3 and 4). These corticomedullary arteries are all of relatively large and nearly uniform size and remain in the corticomedullary interval. If the term arcuate artery is to be employed at all with reference to segments of the renal arterial tree, its use should be confined to the designation of the short curving segments just described, and we propose to use the term in this sense.

From the surface of the arcuate arteries in contact with cortical tissue, arteries of slightly smaller caliber arise at acute angles, inclining slightly into the adjacent cortex. These vessels divide repeatedly but never diverge into the cortex for any great distance from the corticomedullary junction. It is from these vessels that the interlobular arteries arise. These findings conform to the observations of Huber,[14] Traut,[17] and Morison,[32] who have pointed out that there is a series of vessels beyond the arcuate arteries from which the interlobular arteries arise. They opposed the view that interlobular arteries arise from the arcuates directly.[15,33] These vessels immediately beyond the arcuates we propose to call subarcuate arteries (Figs. 4 and 5). They terminate by bending at right angles to their previous course and passing toward the surface of the kidney as interlobular arteries (Fig. 5).

The interlobular arteries are all of small and nearly uniform caliber. They arise from their parent vessel almost at right angles. Those arising from any one vessel are nearly parallel to one another, but tend to diverge slightly (Figs. 5 and 6). Their actual direction in relation to the kidney depends on their position in the renal cortex (Fig. 4). Thus, they may pass proximally toward the lip of the hilus, distally toward the convex border of the kidney, toward the anterior or posterior sur-

face, or toward the cortex of the columns of Bertin. In general, all interlobular arteries supplying the cortex on the surface of the kidney are at right angles to the cortical surface adjacent to them. They are all almost parallel to one another, but, viewed en masse, there is a slight radial arrangement necessitated by the fact that the area of the surface of the pyramids, where the interlobular arteries take their origin, is of smaller extent than the surface of the kidney where they terminate.

At the origin of the interlobular arteries there is often a constriction in the neoprene casts which is occasionally very marked (Fig. 6). A peculiar feature of the interlobular arteries is the maintenance of a nearly uniform caliber almost to the point of termination, this in spite of the fact that they give origin to many afferent arterioles throughout their course and the fact that they frequently divide into two or more branches (Figs. 5 and 6). In most instances the interlobular arteries terminate by breaking up into long afferent arterioles (Figs. 8 and 9). Occasionally they continue on to anastomose with capsular arteries. Such an anastomosis may be of sufficient size to provide an alternate route for the flow of blood to the interlobular artery from which it arises (Fig. 9). Except for such occasional anastomoses the interlobular arteries are end arteries.

The majority of the afferent arterioles arise from the interlobular arteries at right angles, but a few take origin from the arcuate and subarcuate arteries, and pass proximally toward the medulla. The interlobular arteries also produce a few afferent arterioles as they terminate in one or more small vessels directed toward the surface of the kidney (Figs. 8 and 9). The afferent arterioles are of rather uniform length although those taking origin from the larger arteries and those representing the termination of the interlobular arteries are longer than all the other afferent arterioles (Figs. 8 and 10). Some of the afferent arterioles supply two or more glomeruli, rarely as many as five (Fig. 11). The long afferent arterioles arising from the larger arteries supply usually only one glomerulus and never more than two (Fig. 10). Whether the arterioles divide or not, they maintain an almost uniform caliber throughout their length except for the origin and termination where there are constrictions (Figs. 12 and 13). The constriction in the former position, however, is lacking in the arterioles which are formed at the termination of the interlobular arteries (Figs. 12 and 13). The constriction found just where the afferent arterioles enter the glomeruli (Fig. 13) is often hidden from view by the overhanging capillary network of the glomeruli.

It was not possible in our specimens to make a detailed study of the glomerular capillaries.

The efferent arterioles are much narrower than the afferent arterioles (Fig. 14). The majority of efferent arterioles in the greater part of the cortex pursue a short course only, before breaking up into the adjacent peritubular capillary plexus. In this capillary plexus occurs the first anastomosis between the otherwise separate renal vessels. The efferent arterioles adjacent to the pyramids are arranged differently from those of the remainder of the cortex just described and are much wider, being often larger in diameter than the afferent arterioles. They run a short distance toward the pyramids and then abruptly break up into a series of vessels like the lash of a whip. These latter vessels are the arteriolae rectae spuriae (Fig. 15). They present a peculiar and distinctive picture as they stream towards the pelvis. They form bundles of vessels, all of the same caliber, each division maintaining an almost uniform size throughout its length, although dividing to form more vasa recta. These bundles of parallel vessels produce the radiating pattern of the outer portions of the medulla and inner cortex. In our preparations these vessels were not injected sufficiently to enable us to determine whether they would end in a capillary plexus or whether they might skip this plexus and pass directly to veins in the interesting fashion recently described by Trueta *et al.*[9] The arteriolae rectae were injected for distances varying from 0.3 to 2.7 mm., but even the longest failed to connect with vessels of larger caliber or with vessels that in any way resembled veins.

The observations up to this point indicate that the only route of blood supply to the renal cortex is by way of the efferent arterioles draining the glomeruli, while the only blood supply of the medulla is by way of the arteriolae rectae spuriae, draining the glomeruli of the layers of the cortex nearest the medulla.

A careful search for direct vessels to the cortical peritubular capillary plexuses was made while measuring hundreds of afferent arterioles in 10 of the kidneys injected in this study. In the cortex no definite direct vessels were ever encountered. It is true that occasionally a long vessel like an afferent arteriole failed to end in a glomerulus, but it also failed to end in a capillary plexus. It seems probable that such vessels anastomosed with vessels in the capsule of the kidney (Fig. 9). Wherever a suggestion of a direct cortical vessel was found, careful search revealed that it was an efferent arteriole whose glomerulus was hidden behind either large vessels or the capillary plexus. On the other hand, a very few direct vessels of very minute caliber, arising from the interlobular arteries and other large arteries near the medulla, were found which broke up into arteriolae rectae. These are the so-called arteriolae rectae verae. The scarceness, and usually the complete absence, of these direct

vessels in normal kidneys was in marked contrast to the frequency with which such vessels were demonstrated by the same technic in a separate study of the small contracted kidneys of patients suffering from hypertension.

Measurements of Afferent Arterioles and Small Arteries

Our material appeared to offer the opportunity of making a quantitative estimation of the capacity of that portion of the renal vascular bed composed of the afferent arterioles and the interlobular arteries from which the afferent arterioles arise. Accordingly, measurements were made of sprigs of interlobular arteries cut from the neoprene casts. This was done by means of a Zeiss ocular micrometer calibrated against a Zeiss glass millimeter standard scale. From each cast sprigs were selected at random, one or more from the convex border and one or more from the region of the hilus. In the sprigs taken from each of these two regions 50 afferent arterioles were selected and measured after careful inspection in order to include only those arterioles which were free from kinks and which were clearly visible from their origin to the point of entry into the glomerulus. At each of these 100 sites, measurements were made of the length of the afferent arteriole, the width of the lumen of the afferent arteriole at its narrowest point, and the width of the lumen of the parent interlobular artery at the site of origin of the afferent arteriole. An attempt was made to distribute the sites of these measurements evenly along the length of the interlobular arteries. This was accomplished by commencing to measure the afferent arterioles at the termination of an interlobular artery and proceeding with the measurements proximally from this point along the interlobular artery to its origin and then distally along the course of the next adjacent interlobular artery to its termination. In the course of these measurements occasional arterioles arising from the subarcuate arteries were encountered and these arterioles were measured as well as the width of their parent artery. The narrowest point in the width of the afferent arteriole was frequently the constriction described at the point of entrance of the arteriole into the glomerulus and less often the constriction at the origin of the arteriole, but sometimes the narrowest point occurred at other places. When a vessel of arteriolar size divided into two or more afferent arterioles (Fig. 11), the parent arteriole was treated for purposes of measurement as an interlobular artery.

The results of these measurements on the kidneys of one child and 9 adults are shown in Table I. The mean values for the three types of measurements on the 9 adult kidneys were as follows (Table II): length of afferent arteriole, 167.1 μ (standard deviation, 11.6; coefficient of

variability, 6.7 per cent); width of afferent arteriole, 29.8 μ (S.D., 2.8; coefficient of variability, 9.4 per cent), and width of parent interlobular artery, 60.7 μ (S.D., 9.9; coefficient of variability, 16.2 per cent). There was no correlation between any of these measurements and the age of the patient among the 9 adults, but the expected difference from the adult in the length of the afferent arterioles in the child's kidney was noted.

TABLE I

Measurements of Afferent Arterioles and Interlobular Arteries

		Afferent arterioles				Interlobular arteries	
		Length		Width		Width	
Age	Sex	Mean	S.D.	Mean	S.D.	Mean	S.D.
		μ	μ	μ	μ	μ	μ
3	F	108.0	28.0	26.7	3.0	48.0	13.8
20	M	165.6	59.5	27.6	3.4	79.5	32.7
27	M	147.2	40.8	29.7	6.0	55.5	24.6
39	F	183.2	67.2	25.8	8.7	54.6	24.9
43	M	171.2	68.0	30.3	4.2	47.2	21.9
45	M	168.8	62.2	33.9	4.2	60.9	24.0
52	M	157.6	40.1	31.2	12.1	58.2	27.3
54	M	171.2	38.4	28.2	7.7	66.3	33.6
61	F	193.6	47.5	33.3	7.2	71.4	32.1
78	M	145.6	38.4	28.5	10.5	52.8	15.3

As a check on the precision of the micrometer measurements, 100 settings were made at the same site of the width and of the length of an afferent arteriole, and of the width of the interlobular artery at the point from which the afferent arteriole arose, with the results shown in Table III.

DISCUSSION

In our observations made on normal human kidneys, arterioles passing directly from the interlobular arteries to the intertubular capillary plexus of the cortex without first traversing a glomerular capillary tuft (Ludwig arterioles) were never encountered. Neither were any arteriovenous anastomoses like those described by Spanner [26,27] observed. Moreover, arteriolae rectae verae, *i.e.*, arterioles that pass directly from small renal arteries to the medulla without the interposition of a glomerulus, while they did occur, were so rare that it scarcely could be imagined that they play any significant rôle in normal renal physiology. Accordingly, such direct arterioles cannot provide an adequate explanation for the physiologic observations that suggest wide variations in the total renal blood flow,[6,7,10,11] nor could they form an adequate anatomical pathway for the diversion of blood flow from the renal cortex to the medulla recently described by Trueta *et al.*[8,9]

Our observations, therefore, indicate that the fundamental plan of the renal circulation is that originally described by Bowman.[18] The failure of many previous investigators to demonstrate direct vessels

which by-passed the glomeruli in normal kidneys has been attributed by their critics to inadequacy of the injection methods employed. But incompleteness of the injections cannot be admitted as a valid explanation of the absence of direct vessels in neoprene casts, since with this injection material a complete cast of the glomerular capillaries can be made at will and the capillary bed of the parenchyma can be reached with ease. Moreover, the direct vessels of diseased kidneys can be demonstrated without difficulty by this technic.

TABLE II

Summary of Measurements of Nine Adult Kidneys

	Afferent arterioles		Width of interlobular arteries
	Length	Width	
	μ	μ	μ
Mean of 9 adults	167.1	29.8	60.7
S.D.	11.6	2.8	9.9
Coefficient of variability	6.7%	9.4%	16.2%

Having demonstrated the possibility that renal cortical circulation under certain circumstances may be largely diverted from the cortex to flow through an alternative renal medullary by-pass, Trueta *et al.*[9] undertook elaborate studies to demonstrate the structural basis of this functional by-passing mechanism. The anatomical evidence that they were able to assemble led them to conclude that the medullary by-pass is by way of the following structures in sequence: the afferent arterioles

TABLE III

Check on Error in Measurement

	Mean of 100 measurements	S.D.
	μ	μ
Length of afferent arteriole	134.4	13.6
Width of afferent arteriole	29.1	2.1
Width of interlobular arteries	57.9	3.0

of the juxtamedullary glomeruli, the capillary tufts of these glomeruli, their efferent arterioles, the vasa recta derived from them, and thence to the interlobular veins. This is a perfectly clear concept but, unfortunately, there has been a rather widespread misunderstanding regarding the anatomical pathway of the medullary by-pass described by Trueta *et al.*[9] This misconception probably took origin with the appearance of their preliminary paper [8] in which the anatomical pathway was not completely described but only hinted at in vague terms. Indeed, the casual reader might well have been led to believe that the "short-circuiting" of arterial blood through the medulla took place by way of more or less direct arteriovenous anastomoses. This might easily be inferred from such a statement as the following: "The above observations show that

as a result of appropriate nerve-stimulation the blood may be diverted wholly or partly from the cortex and short-circuited through medullary (especially subcortical) blood channels. The large potential capacity of these by-passing channels enables them to transmit the whole of the renal blood inflow whenever the supply to the cortex is diverted and the latter thereby rendered ischaemic." [8] Current editorial comment [34] did nothing to dispel the notion that the medullary by-pass was served by vessels, the existence of which had not previously been generally accepted. Even Fulton, who contributed a foreword to the book subsequently written by Trueta *et al.*,[9] appears to have labored under a misapprehension concerning this point, as indicated by his opening sentence: "Proof of the existence of a vascular shunt capable of cutting off the glomerular circulation of the kidney and reflecting it via the vasa recta directly into the medullary vessels has clarified one of the most perplexing problems of human physiology." On the contrary, Trueta *et al.* stated clearly in the same book [9] that the route of the by-pass is through glomeruli and by way of "channels the existence of which has been known to workers since the time of Bowman (1842)."

In our neoprene preparations it was possible to demonstrate with ease all of the component parts of the medullary by-pass as described by Trueta *et al.*,[9] including variable lengths of the vasa recta. In many instances these vessels were injected through considerable distances and, although they divided repeatedly, we were unable to confirm Trueta's observation that some of these branches turn sharply back toward the arcuate veins that lie in the corticomedullary interval. Our failure to observe such reflex branches could, of course, be attributed to distortion of the flexible neoprene casts when mounted on glass slides under coverslips. However, it is true that the injected vasa recta continued with uniform or diminishing caliber for considerable distances measuring from 0.3 to 2.7 mm. and certainly never showed in our preparations emergence into vessels of increasing diameter that could be regarded as veins. The total length of the medullary by-pass includes, at the least, therefore, the lengths of the afferent and efferent glomerular arterioles as well as of the glomerular capillary loops and in addition the very considerable lengths of the vasa recta that we have demonstrated. Accordingly, while the term "by-pass" is perhaps appropriate, the idea of a "short-circuit" is singularly misleading.

A consideration of the measurements in Table II may make clear their value and their limitations. The mean width and length of the afferent arterioles of the 9 adult kidneys, as shown in Table II, have a coefficient of variability within the normal range of variations of biologic measurements. These mean values would, of course, be a more

valid sample of the width and length of normal afferent renal arterioles of man if a much larger number of kidneys had been examined. However, these measurements provide for the first time the basis for a direct calculation of the capacity of the renal arterioles. It should be pointed out that any such calculation will represent a maximum capacity for this part of the renal vascular bed because, by our technic, all of the vessels, in their relaxed state, are fully distended with neoprene. The repeated measurements of width made along the course of a few interlobular arteries in each kidney permit a calculation of the mean width of the interlobular arteries measured, but do not provide an adequate statistical basis for estimating the mean diameter of all of the interlobular arteries in any given kidney.

Summary

A review of the literature concerning the nature of the fine renal vasculature indicates that most of those who have investigated the renal circulation agree with the conclusion reached many years ago by Bowman. He likened the renal circulation to a portal system, stating that all blood flowing through the kidney passed through two capillary beds (first, the glomerular; and second, the peritubular capillaries) before reaching the renal veins. Only a few students of the renal circulation have seriously challenged this view, and there is now general agreement that any direct vessels that may by-pass glomerular capillaries are so few as to be of no physiologic importance. It is generally accepted that such direct vessels represent the end result of an obliterating process in the glomerular capillary bed that brings into direct connection the afferent and efferent arterioles which together then constitute a direct vessel between the small renal arteries and the parenchymal capillary bed.

Recently it has been shown that there may be a great variation in renal blood flow under varying conditions, and Trueta *et al.* have shown that under some of these conditions the outer renal cortical blood flow may be diverted more or less completely through the inner cortex and medulla. Because the renal circulation time is shorter than normal when the blood is flowing through this so-called "medullary by-pass," there has been speculation as to whether there may be some form of direct communication in the medulla capable of shunting a large quantity of blood more or less directly from the arteries to the veins.

With a view to studying this and other questions regarding the nature of the finer renal arterial vasculature, neoprene casts were prepared by arterial injection and corrosion of 36 normal human kidneys removed at autopsy. Each decade from the first to the eighth inclusive was rep-

resented in the cases from which these kidneys were obtained. Sprigs of the finest structures were mounted in a viscid material on glass slides under a coverslip and studied with the microscope.

In this material only a few direct medullary vessels, *i.e.*, arteriolae rectae verae, were seen. These were far too few to accommodate all of the blood normally flowing through the cortex and, accordingly, it seems most unlikely that such vessels could form the route of the functional by-pass described by Trueta *et al.* Thus, in agreement with Trueta, the only vessels found in our preparations through which blood could circulate in large amounts when diverted from the outer to the inner cortex were those of the juxtamedullary glomeruli, their efferent arterioles and the corresponding vasa recta of the medulla in sequence, all of these being vessels known since the time of Bowman. The exceptional width of the efferent arterioles of the juxtamedullary glomeruli and the number and width of the vasa recta make this a pathway well adapted to carry a large part of the renal cortical blood flow when the medullary by-passing mechanism is in operation.

Measurements were made of the small vessels in the neoprene casts of the kidneys of 9 adults and one child. These measurements should reflect the maximum dimensions of the lumina of the small vessels because the neoprene injected under pressure forms an accurate mould of the vessels fully distended in their relaxed state. The measurements of the width and length of the afferent arterioles provide the basis for making a direct calculation of the maximum capacity of the renal vascular bed at this level.

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DESCRIPTION OF PLATES

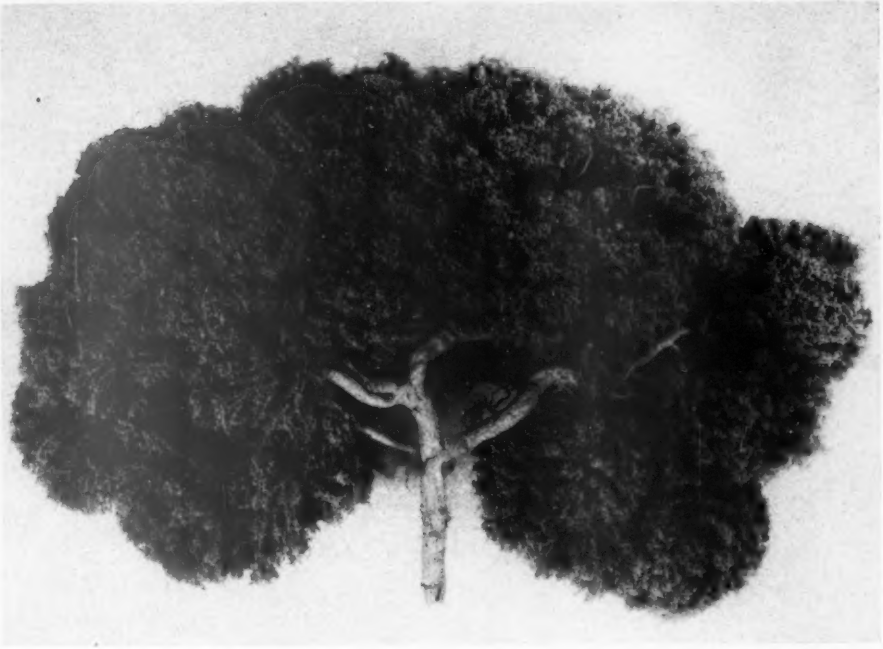
With the exception of Figure 3, all of the illustrations were made from gross or microscopic photographs of neoprene casts of the arterial vasculature of normal human kidneys prepared according to the technic described in the text. It is important to bear in mind in examining the illustrations that one is not viewing the exterior of the arteries, as in an anatomical dissection, but an accurate cast of their lumina.

PLATE 19

FIG. 1. View of the anterior surface of a whole neoprene cast of the kidney. Of note is the division of the main renal artery into a large anterior and a smaller posterior branch.

FIG. 2. A whole neoprene cast of the kidney similar to that in Figure 1 laid open to show the ramifications of the anterior and posterior primary divisions of the renal artery. The two groups of vessels readily fall apart, demonstrating their complete independence. The anterior divisions, on the left, supply considerably more than half of the kidney.

1



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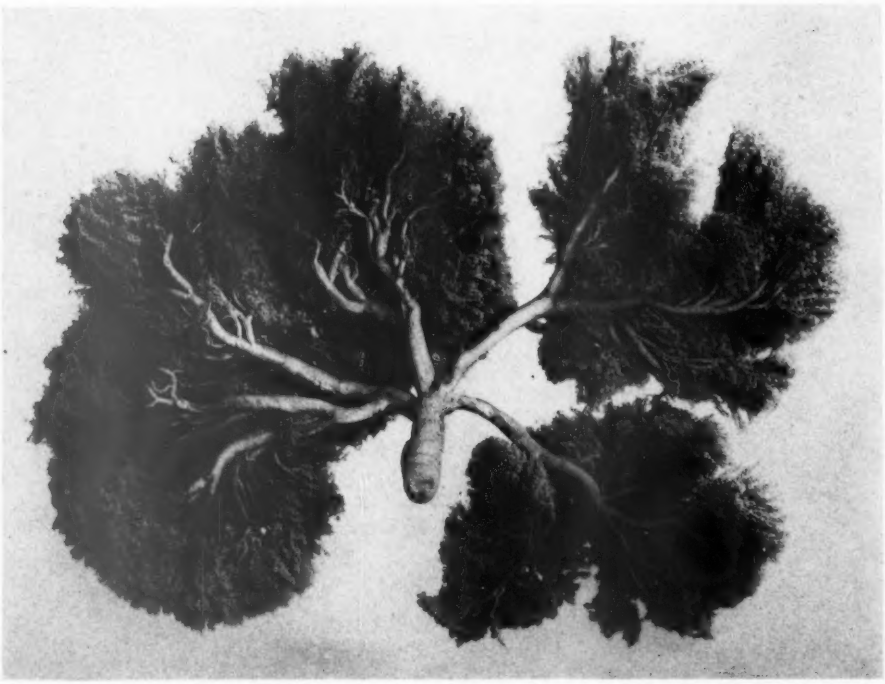


More and Duff Renal Arterial Vasculature in Man

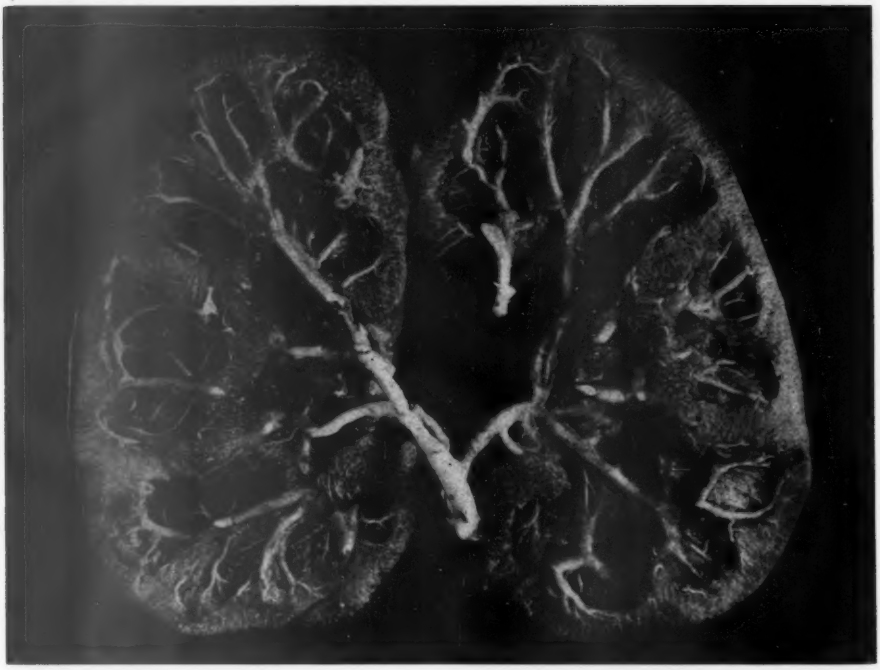
PLATE 20

FIG. 3. Photograph of a kidney injected through the artery with neoprene. The cut surfaces of the fixed and sectioned kidney are shown with the medullary pyramids removed. Some of the spaces match those in the opposite portion of the kidney showing that the two rows of pyramids overlap to some extent in the mid-plane of the kidney. In some places the major branches of the renal artery are seen dividing into groups of large vessels that course between adjacent pyramids. These are the interlobar arteries and it is evident that the divisions of adjacent interlobar arteries entirely surround the cavities left by removal of the medullary pyramids. These branches lying in the corticomedullary interval form gently arching curves that cover the convex surfaces of the pyramids.

FIG. 4. One interlobar artery is shown as it divides into the corticomedullary arcuate arteries of uniform size. The hollowed central portion of the cast corresponds to the position of a part of one medullary pyramid. A series of vessels can be seen which arise from these arcuate vessels and incline slightly into the cortex. These we have referred to as subarcuate arteries. The interlobular arteries arise from these latter vessels, and fan out slightly to supply a rather extensive area of renal cortex.

FIG. 5. A subarcuate artery is shown giving origin to, and terminating in, a series of interlobular arteries. The almost parallel but slightly divergent arrangement of the interlobular arteries can be seen. The uniformity of caliber throughout the length of the interlobular arteries is well shown.

3



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More and Duff Renal Arterial Vasculature in Man

PLATE 21

FIG. 6. Interlobular arteries arising from their parent subarcuate artery at right angles to it. The constriction at the take-off of some of the interlobular arteries may be noted, and also the nearly uniform caliber throughout their length. While the interlobular arteries branch freely, they are clearly "end-arteries."

FIG. 7. A small subarcuate artery terminating in a series of interlobular arteries is shown. Afferent glomerular arterioles are seen arising from the interlobular arteries at fairly regular intervals.

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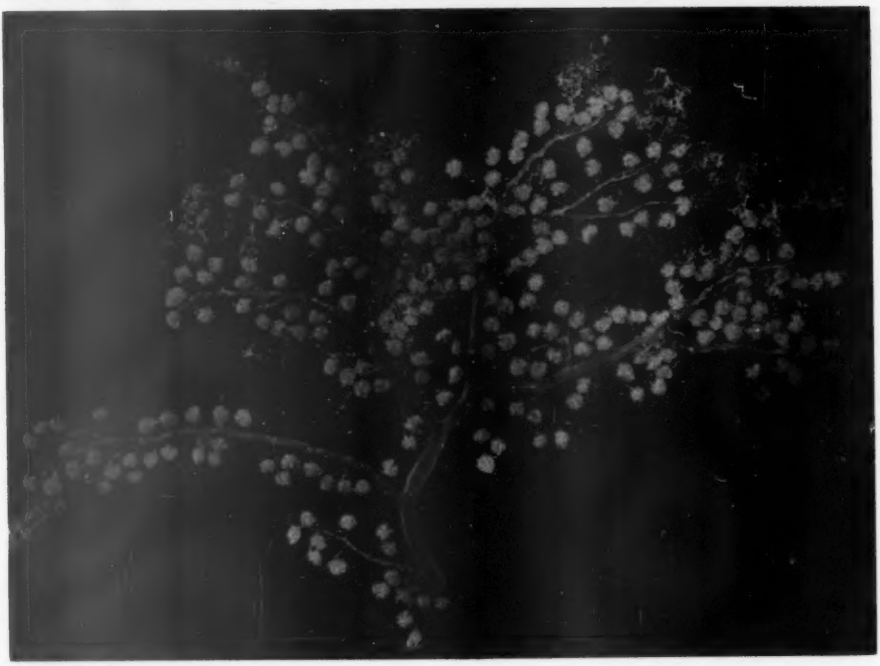


More and Duff Renal Arterial Vasculature in Man

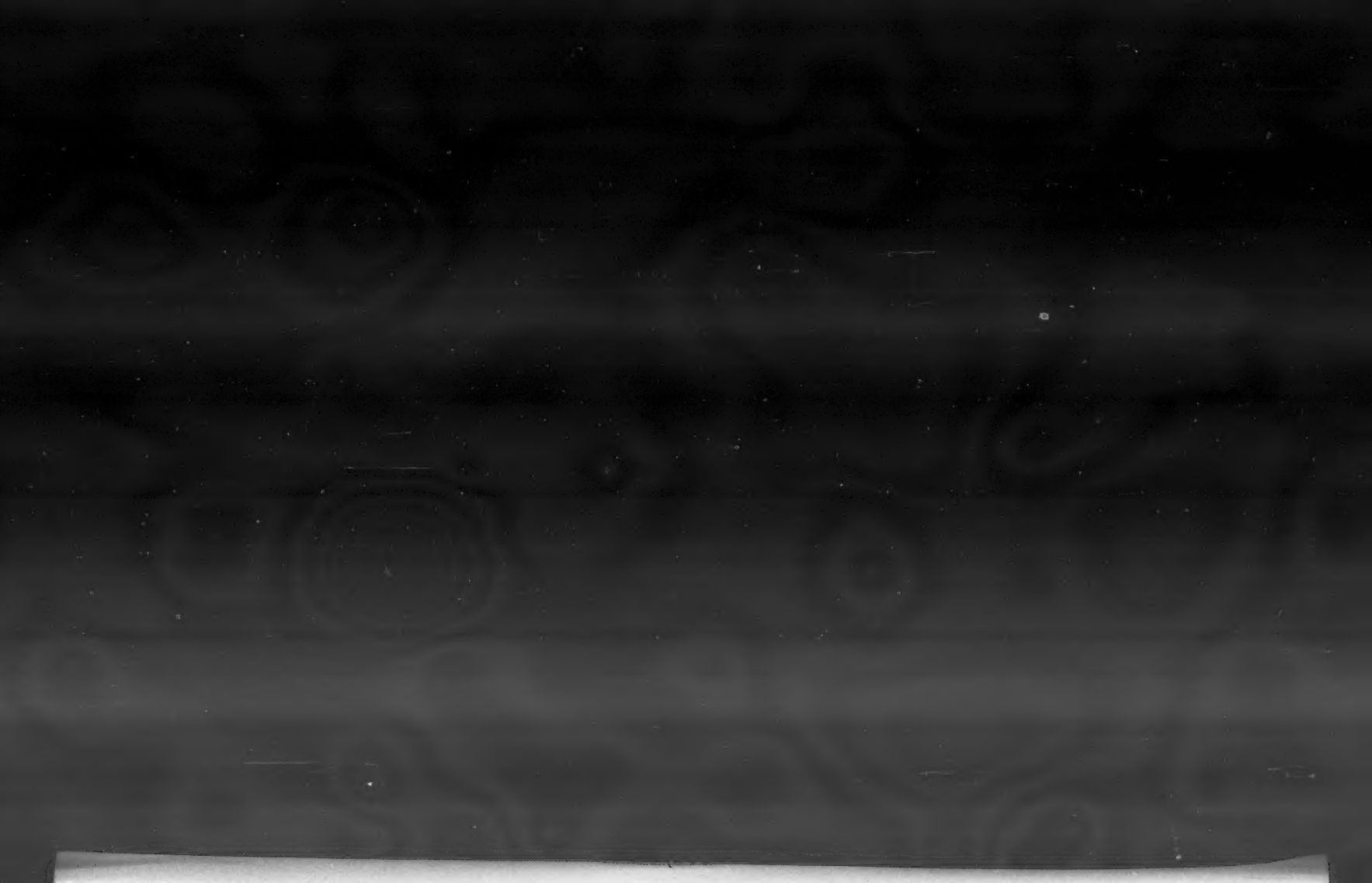
PLATE 22

FIG. 8. An interlobular artery terminating in a series of long afferent arterioles.

FIG. 9. A small vessel is shown arising from an interlobular artery and possibly going to form a major anastomosis with capsular vessels.

FIG. 10. Long afferent arterioles arising from a large subarcuate artery near the corticomedullary interval and each ending in only one glomerulus.

FIG. 11. An afferent arteriole supplying five glomeruli. The caliber is almost uniform throughout the length of this vessel.



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More and Duff Renal Arterial Vasculature in Man

PLATE 23

FIG. 12. An afferent glomerular arteriole showing marked constriction of the lumen at its origin.

FIG. 13. Afferent arterioles showing constriction of their lumina as they enter the glomeruli.

FIG. 14. Three efferent arterioles are shown emerging from behind their glomerular tufts. Note the great discrepancy in size between the efferent and afferent vessels. There are free anastomoses between the capillary beds of adjacent efferent arterioles. There is no constriction at the origin of the afferent arterioles that are formed by the terminal divisions of an interlobular artery.

FIG. 15. Very wide efferent arterioles of glomeruli near the corticomedullary zone are shown giving rise to the vasa recta of the medulla. These latter vessels run a straight and almost parallel course and maintain a uniform caliber almost to their terminations.

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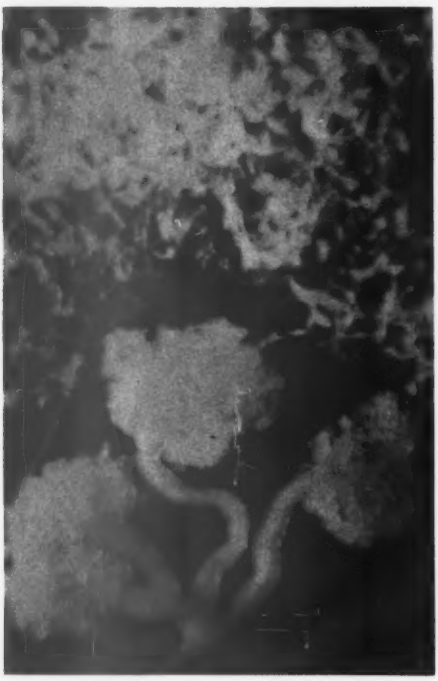

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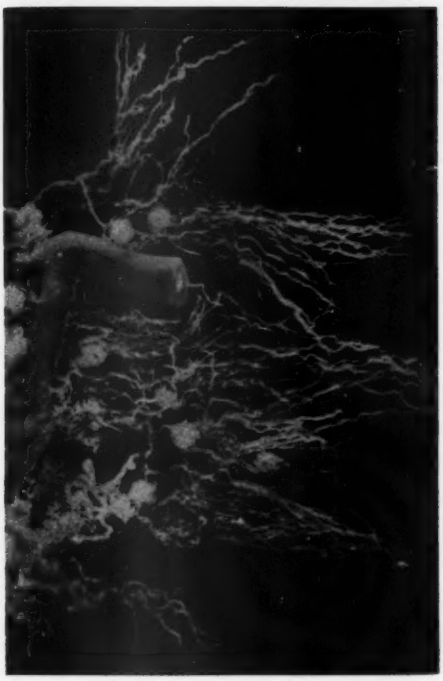

More and Duff Renal Arterial Vasculature in Man

THE TRANSMISSIBLE VENEREAL TUMOR OF THE DOG
Studies Indicating That the Tumor Cells Are Mature End Cells of Reticulo-Endothelial Origin *

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The transmissible venereal tumor of the dog, also known as canine condyloma, venereal granuloma, infectious sarcoma, infectious lymphosarcoma, transmissible lymphosarcoma, and contagious venereal tumor, is a neoplasm occurring naturally on the genitals of both male and female dogs. Tumors thought to be of venereal type have been described also in extragenital locations,[1-6] but transplantation experiments to establish their identity as transmissible venereal tumors have not been performed.

Although the transmissible venereal tumor has been studied by many competent pathologists, the origin and classification of the tumor cells are still subjects of controversy. Earlier workers [7,8] believed that the tumors are composed of epithelial cells and are therefore carcinomas. Others [9-11] considered them to be infectious granulomas. At present, however, there is no serious opposition to the belief that the transmissible tumors are truly neoplastic. Since transmission can be effected only by viable tumor cells, the belief that they are true tumors appears to be justified. As will be shown later, however, behavior of the tumor *in vivo* bears certain similarities to that of infectious granulomas. The consensus [1,12-15] is that the venereal tumors are round cell sarcomas or lymphosarcomas, although certain reservations concerning this concept have been made by some authors. Beebe and Ewing [1] believed the tumor to be either alveolar sarcoma or endothelioma. Feldman [13] formulated the characteristics of the neoplasms which differ from those of most lymphoid tumors and stated that the term lymphosarcoma was used only for morphologic reasons. Stubbs and Furth [15] mentioned that the designation of lymphosarcoma or endothelioma has no sound basis. Kaalund-Jörgensen and Thomsen [16] were impressed with the similarity to reticulo-sarcoma, but thought that further investigation was necessary before including venereal tumors in this group. Jackson [8,17] desig-

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nated the transmissible tumors on the basis of morphologic and cytologic studies as neuroblastoma (sympathogonioma). Mulligan [6] stated that the venereal tumor is apparently a histiocytoma.

The aforementioned investigators have attempted to define the cell type of the tumor by its microscopic structure, and, as is apparent, without any unanimity of opinion. While the cellular character is often the only available means for classifying tumors and establishing their histogenesis, the structure of this transmissible tumor is such that histologic study alone, even by competent observers, is inconclusive in this respect. For this reason we have attempted to determine the cell type of the transmissible venereal tumor by (1) ascertaining the identity of a spontaneously occurring vaginal tumor in a dog as a true transmissible venereal tumor by experimental transplantation to other dogs; (2) vital staining with trypan blue; (3) cytologic and histochemical investigations, and (4) tissue culture studies. Several of these procedures, *e.g.*, certain histochemical and tissue culture studies, have not to our knowledge been employed previously in regard to this tumor. At the same time several hitherto undescribed observations not directly related to the problem at hand have been made. The results obtained reveal that the tumor cells are of reticulo-endothelial origin.

General Data Concerning Transmissible Venereal Tumors

Gross Appearance. Under normal circumstances the transmissible venereal tumors are found as single or multiple, small or large, firm, soft or friable, gray to gray-red, sessile or pedunculated, nodular or papillary masses on the penis and at times on the parietal layer of the prepuce. They occur on the glans, sometimes on the entire penis, at the base of the penis and adjacent prepuce, and may extend to the scrotum and perineal region. In the female the tumors are usually solitary, are found beneath the mucosa in any part of the vagina, often involve the adjoining vestibule, and may spread to the labia. Their size varies from small nodules to large masses and the latter may occlude the vulvovaginal lumen or may protrude between the labia. In both sexes regressive changes are common, so that the tumors may ulcerate and slough, bleed easily, and frequently are associated with a serous, hemorrhagic, or purulent preputial or vaginal discharge.

Microscopic Structure. The tumor cells are large, round, polyhedral or slightly oval, rarely irregular, and display a striking uniformity in size and appearance (Figs. 1 and 2). The nuclei are large, round, relatively vesicular, and contain a prominent, generally single, and eccentrically situated nucleolus. There is an abundant clear or finely granular cytoplasm that stains weakly or pale pink with eosin, or bluish when

the sections are deeply stained with hematoxylin. Specific cytoplasmic granulations (eosinophilic or basophilic granules) are absent with special stains, such as Giemsa's. Mitotic figures are numerous and from 2 to 8 occur in each high-power field. The cells are closely packed and are arranged in diffuse masses (Fig. 1). Throughout the tissue are vascular connective tissue trabeculae of varying size, which sometimes produce pseudo-alveolar arrangements of the tumor cells. The fibrous stroma usually is scanty but is increased in older growths. Connective tissue stains and silver stains reveal an absence of intercellular collagenous and reticulum fibers; the argyrophilic fibers invest groups of cells. Scattered throughout the stroma are small numbers of lymphocytes, macrophages, eosinophils, and tissue mast cells. Polymorphonuclear leukocytes commonly are present in tumors showing regressive and inflammatory changes.

Spread. Although spread may occur by direct extension to adjacent structures and by metastasis to regional lymph nodes and rarely to internal organs, in most cases the tumors are confined to the genitals. The degree of malignancy is relatively slight as the health of the animal is not seriously, if at all, impaired nor is death a sequel. Spontaneous regression is frequent and recurrence is rare even after incomplete surgical removal.

Transmission. The tumors are characterized by the ease with which they can be transmitted to other dogs by natural or artificial means. Under ordinary conditions transmission is effected by coitus, either from male to female or from female to male. Transmission is possible also by an animal licking the affected genitals of another animal and in turn licking its own genitals or those of other susceptible dogs. The neoplasms appear to have a special predilection for the genitals, unless it can be proved that those in extragenital locations are also transmissible venereal tumors.

The experimental transmissibility can be accomplished by injecting emulsions of tumor cells subcutaneously, intraperitoneally, or intravenously; by bringing fresh neoplastic tissue in contact with scarified skin or mucous membranes, or by implanting tumor fragments in the subcutaneous tissue. It is well established that transmission occurs because the transplanted tumor cells are able to grow and multiply in the tissues of the host, and not because of a separate virus or infectious agent.[1,3,12-15] Dogs that recover spontaneously from the natural or experimentally induced disease are immune to attempts at further transmission.

Incidence. The transmissible venereal tumor has a world-wide distribution. During the early part of the twentieth century numerous

cases were encountered in New York City. In the experience of one of us (F. B.) true venereal tumors are at present rare in this region and only two examples were observed in a period of 19 years. It is apparent that in recent years there has been a significant reduction in the incidence, not alone in New York but also in other states.

Material and Methods

Tumor material used for transplantation and subsequent studies was obtained from a spontaneous transmissible tumor, measuring 7 by 11 cm., located in the vagina of an adult German Shepherd. The growth was of several months' duration and was characterized by a serohemorrhagic vaginal discharge and marked bulging of the perineal region. No other symptoms were observed and the animal appeared well. The tumor was removed surgically under general anesthesia on October 2, 1948, and there has been no recurrence to date.

Employing the usual aseptic precautions, small fragments of fresh, intact tumor not exceeding several millimeters in size were implanted subcutaneously, with suturing of the external skin, in the dorsal neck region of 4 male and female dogs of different ages and breeds. This site was selected because of inability of the animals to lick or scratch the operative area, thus aiding in the prevention of secondary infection and trauma. At varying intervals of time after growth had occurred, similar transplantation of neoplastic tissue from the recipient animals was done with 4 additional dogs. In all instances positive takes resulted. The original donor and 7 of the 8 recipient dogs were utilized as sources of tumor for morphologic, cytologic, histochemical, and tissue culture studies.

Morphologic studies were made from tissue fixed in Bouin's solution, Zenker's formalin solution, and 10 per cent solution of neutral formaldehyde, U.S.P. The sections were stained with hematoxylin and eosin, Wilder's reticulum stain,[18] Masson's trichrome stain,[19] Giemsa's stain, and Dominici's stain.[20] Tumor imprints were prepared by gently pressing a clean glass slide upon the cut surface of the growth. These were stained with Wright's and Giemsa's stains.

The following procedures were employed for cytologic and histochemical studies. The Golgi apparatus was identified by the Ludford and Da Fano methods.[21] Mitochondria were stained with the Bensley-Cowdry method [21] and the Mallory phosphotungstic acid hematoxylin technic.[22] Acid and alkaline phosphatases were studied in acetone-fixed tissue by the Gomori technics.[22] In addition, acid phosphatase was identified in fresh tissues (without fixation) by the modification of Bartelmez and Bensley.[23] In frozen sections of formalin-fixed tissue, the

M-Nadi reagent was used for cytochrome oxidase [24] and benzidine and hydrogen peroxide for peroxidase.[25] Lipids were investigated in tumors fixed in a 10 per cent solution of neutral formaldehyde, U.S.P., and in Baker's [26] formol-calcium and formol-calcium-cadmium fixatives. Frozen sections were stained with sudan IV, sudan black, and nile blue sulfate [24,26] and with sudan III.[27] Cholesterol was tested for in sections with the histochemical Schultz technic [21] and the total content in the tumor was estimated by the Liebermann-Burchard method.[28] Baker's [29] acid hematein and pyridine extraction tests for phospholipids were applied to tissues preserved in formol-calcium. Plasmals were investigated by the mercuric chloride-Schiff method [24] and by the dinitrophenylhydrazine method of Albert and Leblond.[30] Unstained frozen sections were observed under polarized light for birefringence. Glycogen was tested for by the periodic acid-Hotchkiss method [31] in picric acid-alcohol-formol and 10 per cent formalin-fixed tissues; glycoproteins, by the periodic acid-Hotchkiss method [31] in formalin-fixed and Zenker's formalin-fixed tissues, and metachromasia with toluidine blue in tissues fixed in 4 per cent lead subacetate.[32]

The procedures employed in tissue culture studies were as follows. Tumor fragments were grown in hanging drop preparations in a medium consisting of two parts chicken blood plasma, two parts dog serum, and one part chick embryo extract. Over 300 fragments were cultured. Some were stained supravitally with either methylene blue, Janus green, or toluidine blue; others were fixed in a 10 per cent solution of neutral formaldehye, U.S.P., and stained with iron hematoxylin, and still others were examined for acid and alkaline phosphatases by the technic of Bartelmez and Bensley.[23] To note the effect of "immune" serum on living neoplastic tissue *in vitro*, a separate experiment was conducted in which 50 tumor fragments were planted in a medium containing "immune" serum. The "immune" serum is so designated because it was obtained from a dog in which a transplanted tumor spontaneously regressed and finally completely disappeared. Many time-lapse photomicrographs were taken.

Vital staining was performed with trypan blue in the eighth animal when the transplanted tumor reached a size of 1.5 cm. A sterile 1 per cent solution of the dye at the dose rate of 1 cc. per lb. of body weight was injected intravenously and repeated in 48 hours. Twenty-four hours after the second injection a small piece of the tumor was removed surgically, from which imprints and sections were made. The tissue was fixed in a 10 per cent solution of neutral formaldehyde, U.S.P., and paraffin sections were counterstained with carmine. Imprints were examined unstained. The same dog then received five additional con-

secutive injections of the dye at 48-hour intervals. Twenty-four hours after the last injection, a small tumor fragment was again removed and treated as before. Another course of five injections of the dye was administered. Forty-eight hours after the last injection, several cubic centimeters of the dye was injected subcutaneously around the tissues of, but not into, the tumor itself. Twenty-four hours later a section of the growth was removed and treated as previously described.

Observations

Transmission Experiments

Following subcutaneous implantation of the tumor, healing occurred by first intention and the sutures were removed in 7 days. In 2 to 3 weeks after transmission there appeared a palpable and sometimes visible spherical nodule from 3 to 6 mm. in diameter. The nodule gradually enlarged so that by the fifth to seventh week it varied from 2 to 4 cm. in size. At this stage the growth projected above the surrounding skin and the covering epithelium appeared reddened and thinned. Shortly afterwards, the covering skin became ulcerated and the tumor broke through, resulting in a discharging necrotic mass. This process was hastened in dogs from which tumor sections were removed for study, despite the exercise of complete aseptic operative technic. In from 3 to 8 weeks after the appearance of regressive changes, there was gradual healing and ultimately a fibrous scar resulted. The latter persisted for several months and could barely be palpated or observed on incision at the end of this time.

Microscopic examination of the early developmental stages of the transmitted tumors revealed structural characteristics identical with those of the original vaginal tumor (Figs. 1 and 2). Tumors undergoing regression exhibited edema, hemorrhage, necrosis, fibroblastic proliferation, and variable numbers of polymorphonuclear leukocytes, lymphocytes, and plasma cells. The covering epithelium was ulcerated and showed pseudo-epitheliomatous hyperplasia at its junction with normal skin. In the end stages there was conspicuous fibrosis with scattered nests of tumor cells. Terminally, no vestiges of tumor cells were seen.

Cellular Characteristics in Imprints

The tumor cells were from two to four times larger than red cells and generally showed a uniform appearance (Fig. 3). They were round or oval and occasionally irregular or polyhedral. The nuclei were spherical or oval, usually eccentrically located in the cytoplasm, and contained a single nucleolus. The nuclear structure was more or less leptochromatic and was made up of fine chromatin granules without a sharp

nuclear membrane. The nuclei comprised about one-half to two-thirds of the entire cell volume. The cytoplasm stained a pale to deeper shade of blue, and the peripheral rim was deep blue in some cells. Specific cytoplasmic granules were absent and small vacuoles occurred in occasional cells. Different stages of karyokinesis were observed frequently. The cells exhibited no evidence of phagocytosis. Also seen were occasional eosinophils, polymorphonuclear leukocytes, tissue mast cells, macrophages, lymphocytes, and plasma cells. Several of these elements, notably the polymorphonuclear leukocytes, macrophages, lymphocytes, and plasma cells, were greatly increased in tumors undergoing regressive changes. Simultaneously, the tumor cells were decreased numerically and often showed marked vacuolization.

The Tumor Cells in the Vitally Stained Animal

Neither in imprints nor in sections was there any visual evidence of granules of dye in the cytoplasm of tumor cells from the vitally stained animal. Diffuse staining of the nucleus and cytoplasm likewise was absent except in dead or dying cells. The cytoplasm of all of the stromal macrophages, however, was very heavily loaded with trypan blue granules.

Cytologic and Histochemical Observations

Golgi Apparatus. The Golgi apparatus appeared as a network adjacent to the eccentric nucleus of the tumor cells (Fig. 4).

Mitochondria. The mitochondria occurred as occasional fine granules scattered throughout the cytoplasm. In the Da Fano preparations for Golgi bodies, minute silver granules, presumably mitochondria, were present in the cytoplasm.

Phosphatases. Fresh unfixed frozen sections showed localization of acid phosphatase in moderate amounts in the nuclei and in traces in the cytoplasm of tumor cells (Fig. 5). No histochemical evidence for either acid phosphatase or alkaline phosphatase was noted in tumor cells which were acetone-fixed.

Oxidases. No cytochrome oxidase or peroxidase activity was seen in the tumor cells. The scattered granulocytes in the sections gave positive reactions for both of these enzymes.

Lipid Substances. Lipid substances were examined in 4 tumors and in all 4 the tumor cells stained faintly with sudan black. In 3 of the tumors no other lipids could be detected with the technics used. The fourth tumor contained, in addition, cytoplasmic lipids occurring as droplets of variable size (Fig. 6). These droplets stained positively with sudan III, sudan IV, and sudan black. The last dye gave the most intense staining reaction. Histochemical tests to characterize the lipids

in more detail were negative. Although the tumor contained 3 mg. of cholesterol per gm., this substance could not be identified histochemically. Phospholipids and plasmals (tissue aldehydes) were not visualized. No pink coloration was observed after staining with nile blue sulfate. Except for the anisotropicity of the stromal collagenous fibers, no anisotropic substances were present.

Miscellaneous. Metachromatic substances, glycogen, glycoproteins, and periodic acid-Schiff positive substances were not identified in the tumor cells.

The Tumor Grown in Vitro

Fragments of tumor grown *in vitro* exhibited a fairly constant behavior pattern. Within 24 hours a less dense zone could be distinguished toward the periphery (Fig. 7). This zone was of considerable width and was caused by a general separation and loosening of the closely packed cells. From the edge of the loose zone, round cells which were undoubtedly tumor cells migrated into the surrounding medium in large numbers (Fig. 8). Also at 24 hours a few spindle-shaped cells could be seen here and there along the periphery. At 48 hours the pattern was one of migration of round cells accompanied by growth and migration of spindle cells (Fig. 8).

With continued cultivation and transplantation of the cultures a pronounced shift in cell population occurred. In the area of new growth the round cells, originally in the majority, could no longer be found. This transformation occurred in from 1 to 3 weeks and upon completion the preparations resembled pure cultures of fibroblasts (Figs. 9 and 10).

The Round (Tumor) Cells. The living round cell as observed with the ordinary microscope and with phase contrast microscopy was quite active. It showed ameboid movements, extended restless tentacles and veils of cytoplasm into the surrounding medium, and sometimes even assumed the characteristics of fibroblasts. Retraction and re-extension of cytoplasmic "feelers" often occurred so actively as to make good photomicrographs difficult to obtain at body temperature (Figs. 11, 12, and 13). The cells exhibited thigmotropism to a relatively high degree. A small piece of hair shaft inadvertently included in a culture was almost completely covered with cells. Spikes of fibroblasts growing out from the parent fragment were commonly surrounded by many round cells. The nucleus was characteristically eccentric in position and was either spherical or ovoid. Occasionally 2 (Fig. 14) and rarely 3 nuclei were found in a single cell. At least one nucleolus was observed in each nucleus, a feature best seen in cells stained with iron hematoxylin. The nucleus showed a strong acid phosphatase reaction (Fig. 14), but little or no alkaline phosphatase activity. The cytoplasm contained so many

fine granules that nuclear identification was often difficult. Supravital staining with Janus green revealed the presence of a few, small, intensely staining, dot-like mitochondria (Fig. 15). The cytoplasm, like the nucleus, gave a strong acid phosphatase reaction (Fig. 14). Attempts to demonstrate the presence of alkaline phosphatase in the cytoplasm were unsuccessful. When cultures were stained with toluidine blue, either a very few or a great many round cells reacted metachromatically, depending upon the age of the culture after the last transplantation. More cells stained as the time interval increased. In different cultures, at the end of 1 day only 10 to 15 cells were stained; at the end of 2 days 100 to 200 were stained; at the end of 3 days it was estimated that about one-fourth of all the round cells stained. The metachromatic material appeared either as blobs or irregularly shaped masses. This reaction was observed only in round cells and in all of these the nuclei stained blue, indicating that the cells were dead.

The Spindle Cells (Fibroblasts). The spindle cells possessed no distinctive characteristics. Occasionally cells with 2 nuclei were observed (Fig. 10). No evidence was obtained that round cells were transformed into spindle cells.

Fate of the Round Cells. The shift in cell population from a mixed mass of many round cells and a few spindle cells to a pure culture of spindle cells was the result of the fact that the round cells degenerate and die more rapidly than they divide. The nuclei showed lysis and the cytoplasm lost its organization. Granular débris of these degenerate and dead cells could be found in the areas of new growth as well as within the parent fragment itself. Concomitant with this round cell disintegration, spindle-shaped fibroblasts grew luxuriantly and ultimately they were the only cells present in the areas of new growth. This transformation occurred within a week or 10 days.

Behavior in "Immune" Serum. Tumor fragments placed in "immune" serum showed no differences in lag phase, rate of round cell migration, rate of growth of fibroblasts, type of growth, type of cells, or general behavior from control fragments placed in normal serum.

Discussion

That the investigations herein described were performed on a true transmissible venereal tumor is proved not only by the morphologic structure, but by the fact of greater importance that the tumor was readily transmitted to other dogs. With the exception of oral papillomatosis, of viral origin, the venereal tumor is the only known transmissible tumor of the canine species. Whether the transmissible venereal tumor occurs in extragenital locations is not within the province

of this discussion, although transmission experiments from these tumors should be done to prove or disprove this contention. Considering the ease with which the venereal tumor can be artificially transmitted, it appears probable that it can occur also in extragenital sites, especially in the cutaneous tissues.

Dry imprints were employed because it is known that there is loss of cellular details in sectioned material and it was hoped that this procedure would be of aid in the recognition of the tumor cell type. Unfortunately, this was not possible as the tumor cell could not be identified positively in the imprints. In any event the appearance of the cells in imprints bore no resemblance to the usual cells seen in smears of blood and in imprints of bone marrow, normal or abnormal. The cells also showed no similarity, in the experience of one of us (F. B.), to neoplastic cells of a large number of canine epithelial and mesenchymal tumors studied by means of dry imprints stained by the Giemsa method. In both imprints and sections the tumor cells maintain their structural type, regardless of whether the tumors occur naturally or are transplanted, and show no evidence of differentiation or dedifferentiation.

Although the cytologic and histochemical procedures were of little aid in ascertaining the origin and classification of the tumor cell, these studies showed that the cells possess more or less specific cytologic and histochemical characteristics.

The well developed Golgi apparatus has been noted also by Jackson.[17] Mitochondria are said to be absent [14] or to stain more weakly than in other cells.[17] In our material, mitochondria were demonstrated in sections with the usual mitochondrial stains and *in vitro* with Janus green, a specific mitochondrial stain. The small silver granules seen in Da Fano preparations are interpreted by many as mitochondria.[21] The evidence therefore indicates that the cytoplasm of the tumor cells contains fine granular mitochondria in sparse numbers and not that they are absent or stain weakly.

Demonstrable lipid droplets are stated to occur in considerable quantities in the tumor cells.[14,17] This was found to be true in only one transmitted tumor in which sudanophilic lipid globules were localized in the cytoplasm. In three other tumors lipid droplets were absent, but because of the faint staining with sudan black it is believed that lipids are present and they are interpreted as "bound lipids." As the tumor with lipid droplets microscopically showed degenerative changes of its cells, it is possible that the visible lipids simply indicated fatty metamorphosis. Further attempts to characterize the lipids histochemically were unsuccessful, although it is known that they are not doubly refrac-

tive and the absence of a pink coloration with nile blue sulfate signifies that neutral fats (nonsaturated glycerides) are not present. The inability to identify cholesterol histochemically, despite its occurrence in the tumor, is probably due to the fact that this substance is not concentrated in sufficient amounts in restricted foci.

A positive acid phosphatase reaction was demonstrated in the nuclei and cytoplasm of tumor cells in both tissue cultures and in sections, but, however, in fresh unfixed tissue only and not in acetone-fixed tissue. This is explained by the fact that from 70 to 95 per cent of the enzyme is lost during fixation and embedding.[33]

The presence of metachromatic substances in the tumor cells *in vitro* is difficult to evaluate and explain. The cultivated tumor cells showing metachromasia were entirely different in cultural characteristics from either normal or tumor mast cells.[34] In cultures only tumor cells and fibroblasts grew, but other cellular elements of the tumor, including tissue mast cells, did not. The complete absence of metachromatic granules in tumor cells of sections and imprints, whether of young, old, or degenerating natural or transplanted tumors, proves that the cells are not mast cells. It is true[35] that in immature mast cell neoplasms of the dog, anaplastic tumor cells have a greatly reduced mast granule content, although the granules can always be demonstrated in suitably fixed and stained sections as well as in imprints and *in vitro*.[34] Since the degree of metachromasia was associated with ageing of the cultures, it appears that this change is one either of degeneration or senility of the tumor cells.

Crile and Beebe[36] found that when whole blood from dogs that had recovered spontaneously from the transmissible venereal tumor is transfused into animals with active tumors, either a cure or regression of the growth was effected in the recipient. One might therefore expect that growth *in vitro* would be interfered with or non-existent when serum from an immune animal is used in the culture medium. However, no cultural differences existed in tumor grown in "immune" and in normal serum. Apparently the conditions *in vivo* and *in vitro* are different in respect to the reaction of the tumor cells to "immune" serum.

With the exception of the time element, the behavior of the tumor *in vivo* and *in vitro* showed certain similarities. In the tissues of the host there is, at first, growth of the tumor cells which is then followed by regressive changes of the cells and a proliferation of fibroblasts. This process from the initial transplantation occupies a period of from 2 to 4 months and may be accelerated by surgical procedures and secondary infection. Similar changes may occur in spontaneous tumors

located on the genitals, although in these instances fibrous replacement may be only partial or requires greatly extended periods of time. A probable explanation for the delayed involution of spontaneous tumors is that the genital tissues are the natural sites for the development of the tumor and that conditions here are most suitable for the maintenance of growth. In transplanted extragenital subcutaneous locations, on the other hand, even though initial growth occurs it appears that optimal conditions to maintain this growth are not present, so that a relatively early spontaneous regression takes place.

In tissue culture the round tumor cells fail to multiply to a degree sufficient to maintain themselves and they disappear in from 1 to 3 weeks. The shift in cell population from many round cells and a few fibroblasts to practically a pure culture of fibroblasts suggests a plausible explanation for the regression of the tumors *in vivo*. The unusual feature in both circumstances is the eventual replacement of the tumor cells by fibroblasts, very rapidly *in vitro*, more slowly in transplanted extragenital subcutaneous sites, and partially or greatly delayed in spontaneous genital locations. The inability of the tumor cells to maintain themselves *in vitro* may be due to the fact that they are mature end cells and thus unable to undergo further growth, and that the environment in tissue culture is lacking in specific substances present in the living tissue essential for their optimal growth.

With respect to the behavior of the tumor cells in tissue culture, they resemble macrophages of the type seen in normal cultures, *i.e.*, the wandering cell which is isolated, rounded, and exhibits active pinocytosis. In structure and behavior they appear and act like the epithelioid cells which were cultured and photographed by Warren H. and Margaret R. Lewis in their film of "Normal and Abnormal White Blood Cells in Tissue Culture." However, certain criteria considered characteristic of macrophages, such as phagocytosis and the ability to store acid vital dyes, were not exhibited by the tumor cells. In sections and imprints a careful search indicated no evidence of phagocytic activity by the tumor cells, even in areas of hemorrhage and necrosis. Intensive and prolonged vital staining of the host with trypan blue, together with injection of the dye into the tissues immediately surrounding the tumor, revealed no segregation of the dye in the cytoplasm of the tumor cells. In human histiocytomas the tumor cells are phagocytic as shown by their ability to be vitally stained with colloidal iron.[37] Therefore, despite the fact that the tumor cells *in vitro* resemble macrophages in structure and behavior, they do not possess certain properties considered characteristic of the latter elements *in vivo*. When one recalls, in addition,

that the tumor cells show no evidence of differentiation or dedifferentiation, that they are unable to maintain themselves *in vitro* for more than a week or so, and that spontaneous regression occurs *in vivo*, the conclusion is probable that the tumor cells are mature definitive cells. As such, they are unable to show unlimited growth, despite their active appearance as judged by frequent mitotic figures. This concept is supported by the relatively limited growth potentialities, once growth has begun, that are observed both *in vivo* and *in vitro*. Since the cultured cells show structural and behavioristic patterns similar to those of cultured macrophages, even though they do not morphologically resemble macrophages in sections and in imprints, an origin from the reticulo-endothelium is suggested. This assumption seems appropriate because the only visible, conveniently available means for observing the reactions of the living cell is *in vitro*, albeit in an acknowledged artificial environment. In culture the mature definitive cells manifested some physical attributes of their assumed ancestral prototype, namely, the macrophage, of reticulo-endothelial genesis. This concept appears all the more justified when it is considered that we are dealing here with a tumor unique in many respects. Following its original transformation from the reticulo-endothelium, the mature definitive cell has apparently lost its original morphologic and physiologic characteristics as they can be observed in sections and imprints, so that its origin is completely obscured.

On the assumption that the tumor cells are mature end cells of reticulo-endothelial origin, how should the transmissible venereal tumors be correctly designated? Carcinoma is obviously incorrect since the cells are not epithelial. Lymphosarcoma is likewise inappropriate as the cells do not belong to the lymphoid series. Sarcoma in its broadest sense is less objectionable because the cells are of mesenchymal type, although no exact information is conveyed concerning the cell type. Reticulum cell sarcoma has perhaps some histogenetic justification, but not on a morphologic basis. Neuroblastoma is manifestly incorrect and requires no further comment. Histiocytoma is inappropriate because the cells do not exhibit the morphologic and physiologic attributes of histiocytes, and, furthermore, the transmissible tumor is entirely different structurally from human histiocytoma. Since the naming of the cell type of the tumor offers an unusual problem in oncology, obviously some new term not previously used would have to be formulated. This is perhaps not really necessary as changes in medical nomenclature generally meet with considerable resistance and may complicate an already confused situation. It is therefore suggested that the designa-

tion of "transmissible venereal tumor" be retained, with the knowledge that the tumor cells have the morphologic and functional characteristics of mature end cells of reticulo-endothelial origin.

Summary and Conclusions

Previous attempts to classify the cell type of the transmissible venereal tumor of the dog have been based only on histologic examination. As a result the tumors have been designated by a variety of names with little unanimity of opinion. Our investigations, utilizing transmission, morphologic, cytologic, histochemical, and tissue culture studies, reveal that the tumor cell is a mature end cell of reticulo-endothelial origin. Instead of formulating a new name for the tumor, it is suggested that the designation "transmissible venereal tumor" be retained.

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DESCRIPTION OF PLATES

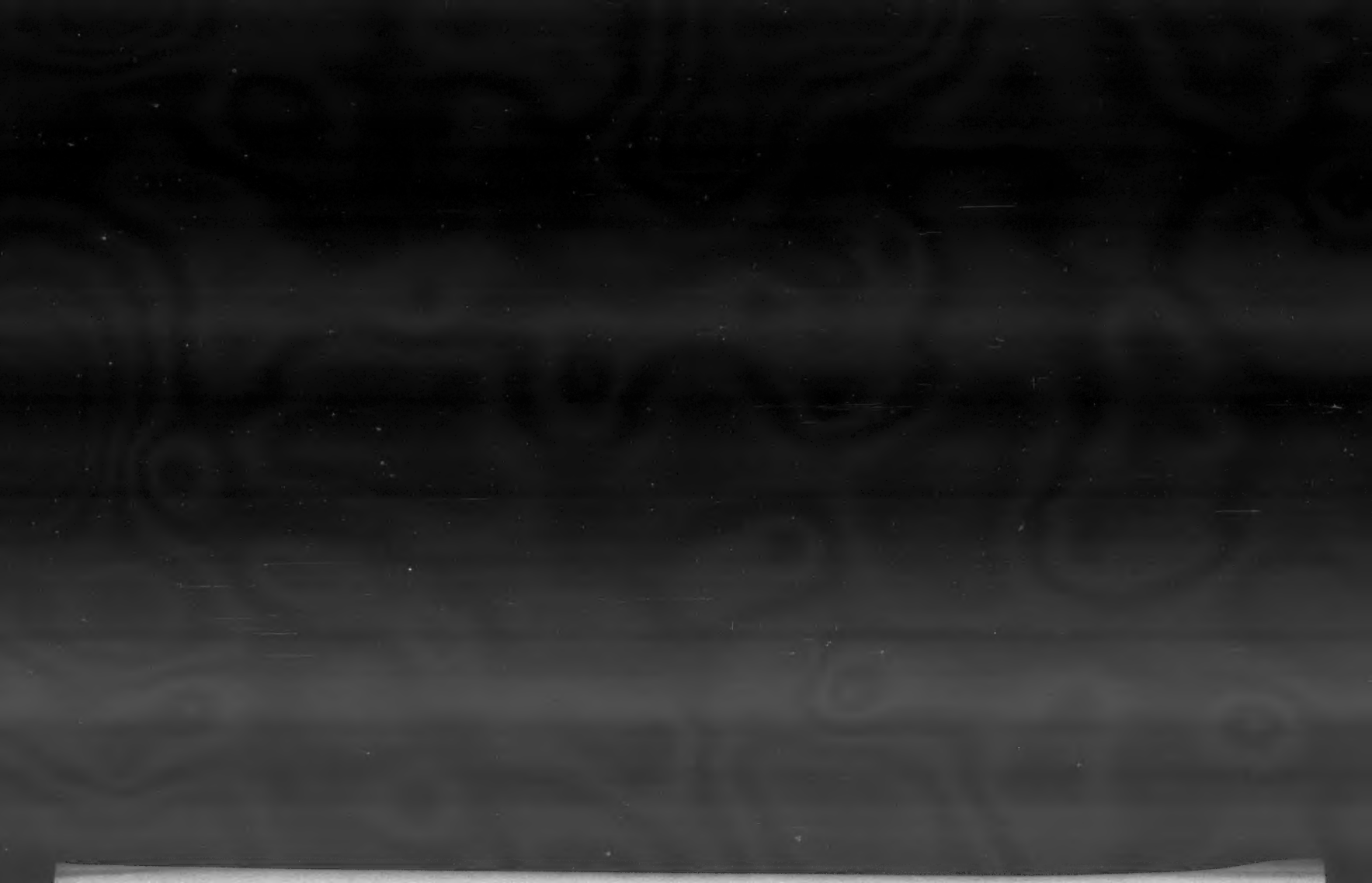
PLATE 24

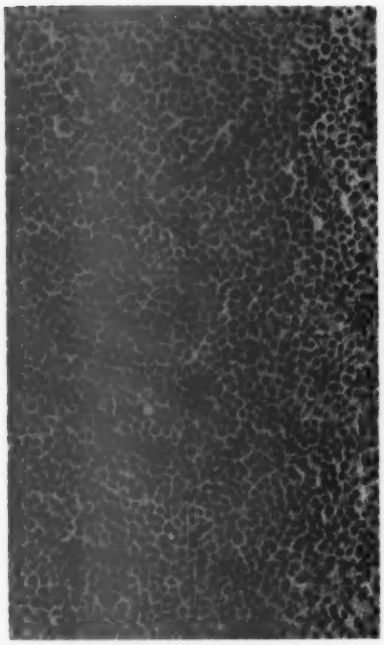
FIG. 1. Low-power view showing general structure of the canine transmissible venereal tumor. Hematoxylin and eosin stain. × 200.

FIG. 2. High-power view of the cellular structure. Of note are the mitotic figures. Hematoxylin and eosin stain. × 900.

FIG. 3. Imprint of a mature, regressing, transplanted venereal tumor. Cytoplasmic vacuolization and two polymorphonuclear leukocytes may be observed. Wright's stain. × 900.

FIG. 4. Photomicrograph of the Golgi apparatus adjacent to the slightly eccentric nucleus. Ludford technic,[21] no counterstain. × 900. The inset shows the Golgi apparatus at a higher magnification. × 2700.





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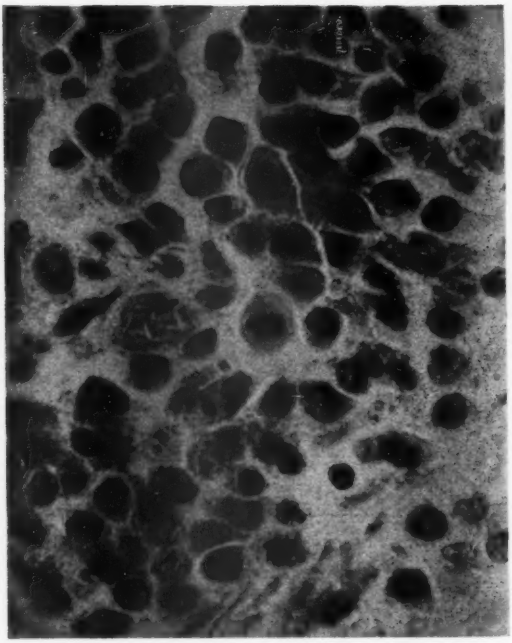

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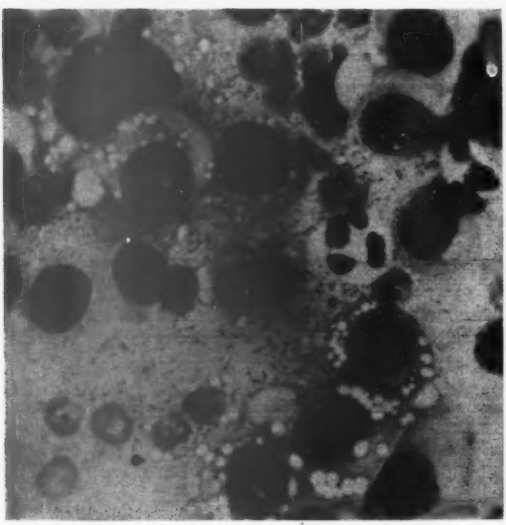

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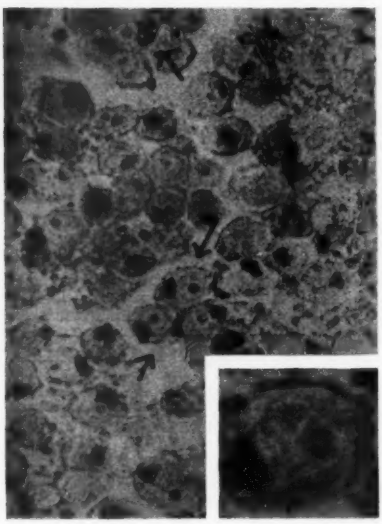

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PLATE 25

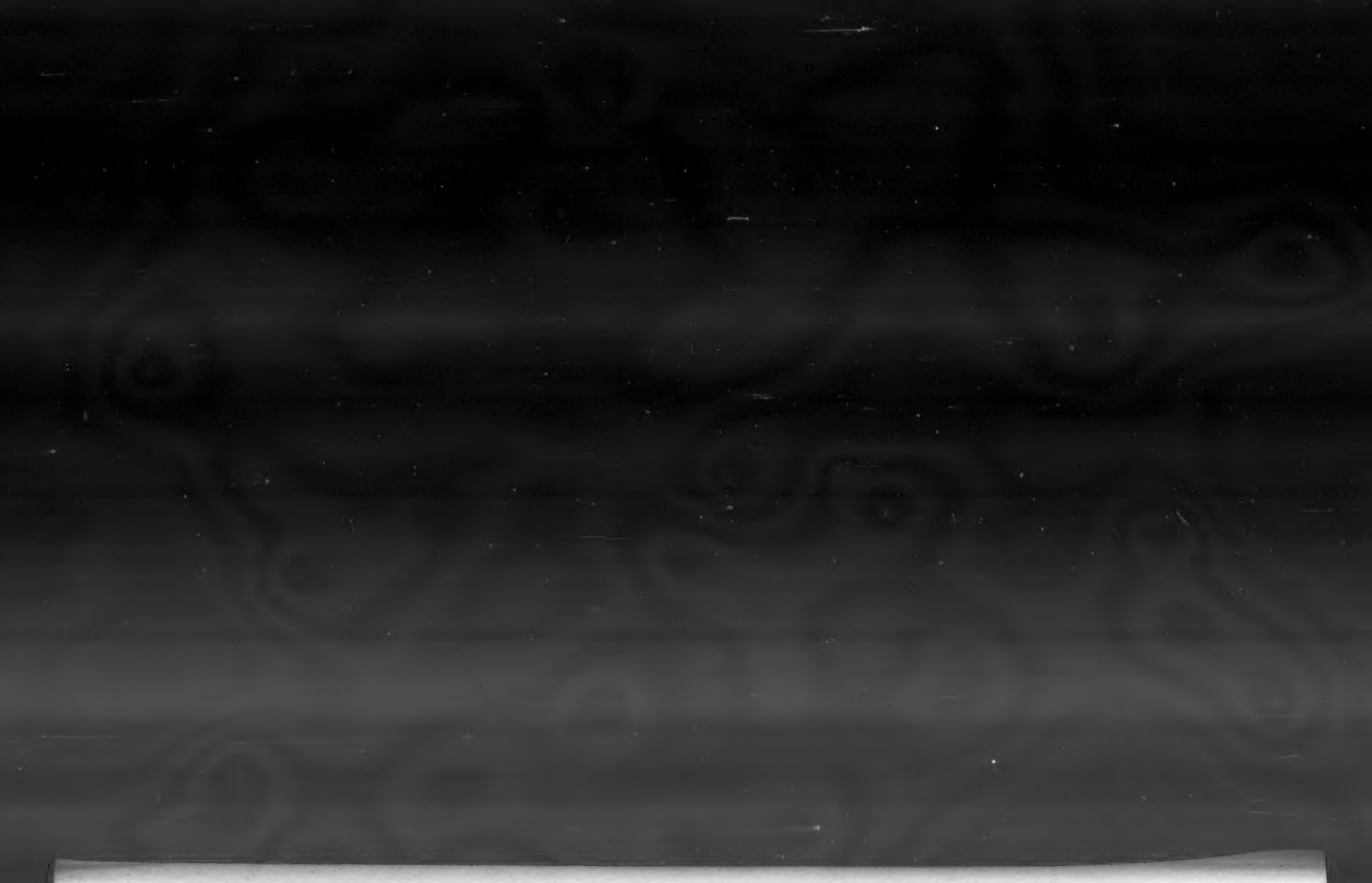
FIG. 5. Acid phosphatase in tumor cells. The reaction is more intense in the nucleus than in the cytoplasm. The variability between the reactions in different cells is due in part to the thickness of the section. Enzyme was detected in unfixed frozen sections (25 μ thick) according to the method of Bartelmez and Bensley.[23] No counterstain. × 900.

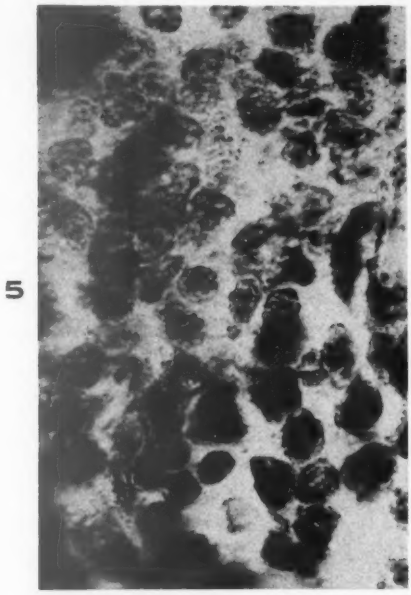
FIG. 6. Lipids in the form of droplets in tumor cells of a regressing tumor. A thick frozen section was fixed in formol-calcium and stained with sudan black. No counterstain. × 900.

FIG. 7. Peripheral zone of a living unstained tumor fragment cultured for 24 hours. This shows the beginning of separation and migration of the round tumor cells. × 200.

FIG. 8. Same tumor fragment as seen in Figure 7 at the end of 48 hours of cultivation. The clearer zone is due to further separation and migration of round cells. The appearance of fibroblasts may be noted. × 100.

FIG. 9. Same culture as shown in Figures 7 and 8 but 2 weeks later. The round cells have completely disappeared and only fibroblasts are present. The shift in cell population is complete. Acid phosphatase stain. × 200.





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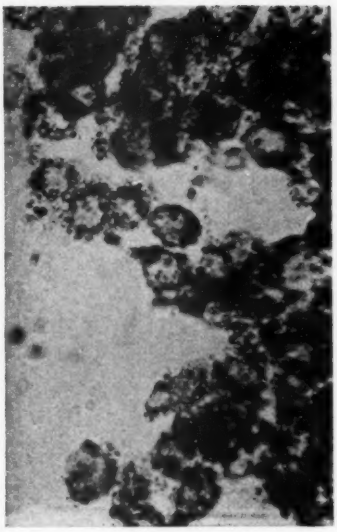

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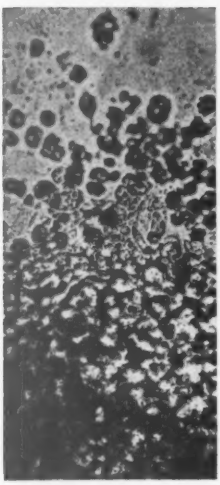

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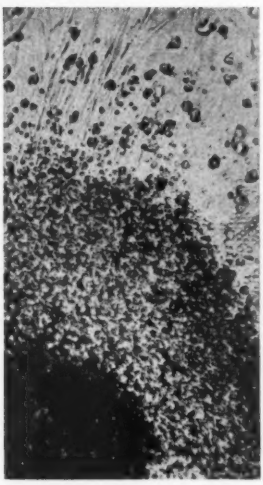

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PLATE 26

FIG. 10. Living, unstained, binucleated fibroblast from a tumor fragment cultivated for 3 weeks. Phase contrast. × 1455.

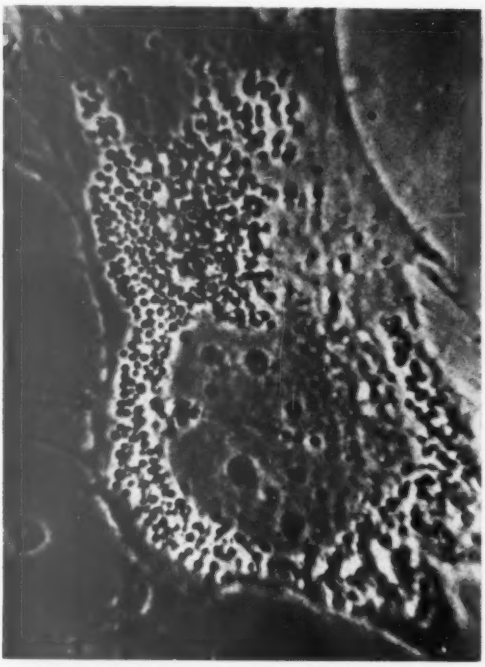
FIG. 11. Living, unstained round cell extending a large process into the surrounding medium. Three-day-old culture. Phase contrast. × 970.

FIG. 12. Living, unstained round cells exhibiting ameboid movement. The cell to the right is the same as the one in Figure 11. Three-day-old culture. Phase contrast. × 970.

FIG. 13. Living, unstained round cell showing pinocytosis. Three-day-old culture. Phase contrast. × 970.

FIG. 14. Binucleated round cell stained for acid phosphatase. Two-day-old culture. × 970.

FIG. 15. Round cell with dot-like granular mitochondria, a few of which stain intensely, in the cytoplasm. Supravital staining with Janus green of a 3-day-old culture. × 970.



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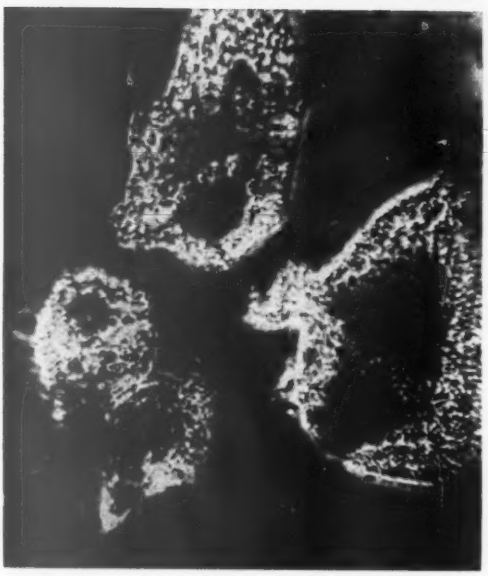

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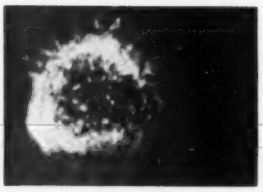

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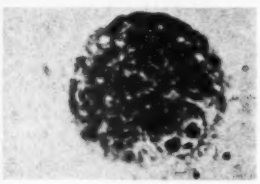

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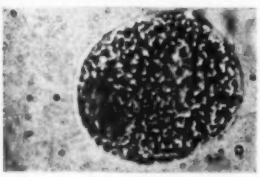

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Venereal Tumor of the Dog

ACID MUCOPOLYSACCHARIDE IN DEGENERATIVE DISEASE OF CONNECTIVE TISSUE, WITH SPECIAL REFERENCE TO SEROUS INFLAMMATION *

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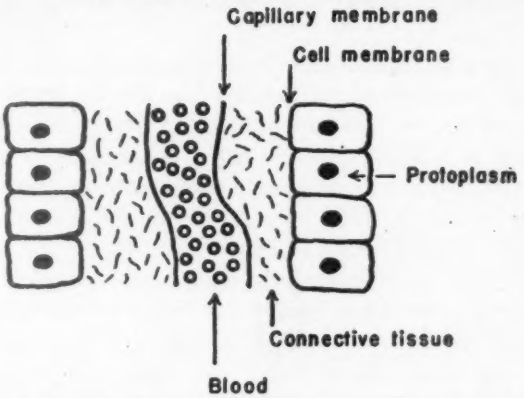
The accumulation of acid mucopolysaccharides in certain lesions of rheumatic diseases was noted in a previous study.[1] Further experience has indicated the significance of this polysaccharide formation, particularly in regard to chronic serous inflammation. It is the purpose of this report, therefore, to present these findings in association with a brief review of the concept of serous inflammation. It is also intended to re-emphasize the relationship of this tissue reaction to certain current



Text-Figure 1. The anatomical components of the hematoparenchymal system. Modified from Schade.[2]

investigations. Because a detailed consideration is impractical here, only the general theory and a few examples will be discussed. Local morphologic applications will be reported elsewhere.

The term "serous inflammation" is used to designate certain phenomena that affect the hematoparenchymal system. Text-Figure 1 modified from Schade's text [2] illustrates the relationship of the principal anatomical components. To reach a parenchymal cell from the blood stream a substance must pass through the capillary wall, the connective tissue, and the cell membrane. Likewise, a cellular product must pass through the same elements to reach the vascular or lymphatic circulation.

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Various authors have stressed the alterations of each of the morphologic elements in these tissue changes. Eppinger [3,4] and his school, and Schürmann and MacMahon,[5] for example, emphasized the endothelial abnormalities (dysorie); Schade [2] stressed the connective tissue reactions, and others have been concerned with dysfunctions of the cell membranes.

According to the theory of serous inflammation as proposed by Rössle [6] and elaborated by Eppinger,[3,4] damage to any of the constituents of the hematoparenchymal system may be followed by a relatively cell-free edema. The accumulation of this protein-rich fluid in the connective tissue with its consequent separation of the capillary from the parenchyme supposedly impairs the transport of nutritive materials and cellular metabolites. This, then, is said to result in depression of cellular respiration.

Since the alterative and reactive processes may vary in severity and chronicity, observable lesions will differ. In the milder forms edema alone may be noted, whereas in the more severe varieties necrobiotic alterations of connective tissue and blood vessels as well as of the parenchyme may occur. Following mild transient injury there may be complete restitution, while lesions of longer duration ultimately result in sclerosis of blood vessels and connective tissue.

Because the exact chronologic order of the chemical and physical alterations is not certain, part of the above considerations are hypothetical and disagreement exists with regard to certain etiologic, pathogenetic, and nosologic aspects.[6-8] In contrast, descriptions of the morphologic alterations, particularly in the later stages of the disease process, are quite generally accepted.

Chronic serous inflammation is considered to be of widespread significance, particularly as it pertains to rheumatic lesions, sclerosis of blood vessels, vascular alterations that accompany malignant hypertension, certain forms of nephritis, a group of neural lesions characterized by increased interstitial connective tissue, and hepatic alterations associated with hyperthyroidism.

Eppinger [3,4] and his school utilized allyl formate, thyroxin, chronic fatigue, oxygen lack, and hypersensitivity reactions to produce serous inflammation experimentally and, therefore, considered such lesions to represent tissue reactions to certain non-specific damaging agents.

Since the accumulation of acid mucopolysaccharides is readily demonstrable in many serous inflammatory alterations, it became of interest to determine whether they are generally formed in these processes.

Materials and Methods

The material examined was as follows:

Material Studied

I. Autopsy and surgical tissues
  A. Classified as to definitive lesion
    1. Fibrinoid (Rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus, bursae, "ganglia," peptic ulcers, placentae)
    2. Sclerosis (Arteriosclerosis, scleroderma, sclerosis of renal medullary substrate)
    3. Amyloid (Primary or secondary amyloidosis, amyloidosis associated with multiple myeloma, and local or tumor amyloidosis)
    4. Hyalin (Granulation tissue, healing wounds, foreign body reactions, inflammatory processes surrounding abscesses)
  B. Classified as to etiology
    1. Hormonal influences (Myxedema, hyperthyroidism, diabetes, pituitary basophilism, Addison's disease, acromegaly, decidual tissues, gynecomastia)
    2. Bacterial infections (Diphtheria, pyogenic infection, tuberculosis)
    3. Physical agents (Lesions resulting from roentgen radiation, mechanical trauma, cold, heat, anoxia)
    4. Tumors (Benign: myxomas, fibromas, fibro-adenomas of breast, neurofibromas. Malignant: squamous cell carcinomas, basal cell carcinomas, fibrosarcomas, myxosarcomas, liposarcomas)
  C. Classified as to anatomical source
    1. Skin lesions (Scleredema, scleroderma, pretibial myxedema, balanitis xerotica obliterans, necrobiosis lipoidica diabeticorum, granuloma annulare, disseminated lupus erythematosus)
    2. Nerve lesions (Primary nerve alterations: interstitial hypertrophic neuritis, neurofibromatosis. Nerve alterations associated with other conditions: systemic amyloidosis, non-specific inflammatory neuritis, hypertensive neuropathy)
    3. Cardiovascular (Disseminated connective tissue diseases: rheumatic fever, rheumatoid arthritis. Fetal endocarditis, thrombo-angiitis obliterans, idiopathic medial necrosis of aorta)
    4. Pulmonary (Interstitial pneumonitis, primary and secondary; pulmonary arteriosclerosis; pleuritis associated with disseminated lupus)

II. Experimental
  A. Hypersensitivity reactions
    1. Bacterial type (Tuberculin reactions)
    2. Anaphylactic type (Horse serum)
  B. Hormonal
    1. Desoxycorticosterone acetate *
    2. Lyophilized anterior pituitary extract *
    3. Cockscombs
  C. Viruses (Influenza and Newcastle viruses on chorioallantoic membranes)
  D. Nutritional (Scorbutic guinea-pigs, rachitic rats, rats deficient in biotin, B_6, or pyridoxine)
  E. Miscellaneous
    1. Rat and chick embryos
    2. Tadpoles

* Treated animals obtained from Dr. Hans Selye.

The material was fixed in formalin for 24 to 48 hours and embedded in paraffin. Sections were cut at 6 to 8 μ and stained with 1 per cent toluidine blue, crystal violet, van Gieson's stain, and phosphotungstic acid hematoxylin after chromium chloride mordanting.[9] The periodic acid leukofuchsin method as used by McManus [10] and Hotchkiss [11] was employed to demonstrate polysaccharide aldehyde. Control sections for plasmal were run.[12] Desoxyribonucleic acid was demonstrated by the method of Feulgen as applied by Stowell [13] and, on selected sections, the Sakaguchi reaction for arginine as modified by Weber [14] and by Serra [15] was employed. When metachromasia was demonstrated with toluidine blue, representative sections were incubated with hyaluronidase (testis) in saline solution, using saline solution and albumin in saline solution as controls.[1]

The interpretation of these staining reactions has been discussed previously.[1] We share the opinion of Meyer [16] that any substance of large molecular weight with free acid radicals may induce metachromasia. Since the chromotropic substances formed in serous inflammation, however, largely occur extracellularly (mast cells may be present also in increased numbers), it has been assumed that they represent acid mucopolysaccharides.[17,18] This is supported by certain ancillary observations. For example, structures from which acid mucopolysaccharides can be extracted and identified by chemical means stain metachromatically with toluidine blue. In some instances, too, incubation of the tissue sections with hyaluronidase prior to staining prevents the development of metachromasia, and in some sections that show metachromasia it is possible also to obtain positive reactions for polysaccharide aldehyde and glucosamine.

Experimental Results

(a) Acid mucopolysaccharides are frequently formed in subacute and chronic serous inflammation. (b) Acid mucopolysaccharide formation cannot be correlated with any particular extrinsic agent. The study reported here indicates that acid mucopolysaccharides occur quite frequently in the later stages of serous inflammation. For example, these polysaccharides are easily demonstrated in lesions associated with rheumatic fever, rheumatoid arthritis, and disseminated lupus erythematosus; in patients with diabetes, myxedema, and pregnancy; after prolonged administration of estrogens, androgens, desoxycorticosterone or lyophilized anterior pituitary extract; in hypersensitivity reactions; at the periphery of inflammatory lesions caused by bacteria or viruses; and after exposure to x-radiation and cold, trauma, and anoxia. In early mild injuries or after extremely severe damage, however, acid mucopolysaccharide may not be demonstrable by the methods utilized in this

study. In very old lesions, too, the staining reactions are somewhat inconstant.

(c) Various etiologic agents may have either a generalized or local effect. Some irritative or stimulating mechanisms are characterized by generalized serous alteration. This is illustrated by the changes that occur in myxedema, diabetes, and after administration of desoxycorticosterone. In other instances the alterations are purely local as, *e.g.*, in chronic inflammation of bursae. In part, of course, this is explainable by the local action of the damaging agent. Special tissue susceptibility to an etiologic agent, however, must be postulated in some cases. When androgens are administered to a caponized cock, the increase in the size of the comb is accompanied by the accumulation of metachromatic material.[19] This alteration, of course, is out of proportion to the response by other tissues.

(d) Tissues vary in their ability to form acid mucopolysaccharide. *(e) Acid mucopolysaccharide may occur in tissues or lesions whose energy metabolism is characterized by increased glycolysis.* The facility with which acid mucopolysaccharide formation may be induced also seems to depend in part upon the amount of mesenchyme normally present in the tissue studied. For example, it is difficult to demonstrate acid mucopolysaccharide accumulations in liver, adrenal, or spleen, but the polysaccharides are formed readily in renal medulla, cartilage, synovia, and serous surfaces. This is in accord with previous observations. The latter tissues, which have a relatively poor vascular supply, have been called bradyotrophic, and their propensity to produce metachromatic substances is well known.[20] It has been found that the energy metabolism of bradyotrophic tissues is characterized by increased glycolysis.[21-24] Neoplastic,[21,25,26] embryonal,[27] and inflamed [28] tissues share this metabolic characteristic, and they too may contain increased quantities of acid mucopolysaccharides.

It is apparent, however, that increased glycolysis is not the only factor involved in the synthesis of acid mucopolysaccharides, for only certain tissues (connective tissue, glands, and mucous surfaces) are able to form them. Other tissues (brain, for example), although actively glycolysing, do not produce them. The nature of the enzymes, substrate, and reaction conditions for acid mucopolysaccharide formation is not known.

(f) Acid mucopolysaccharides may participate in the formation of fibrinoid, sclerotic, hyaline, and amyloid material, and connective tissue fibers. The possibility that acid mucopolysaccharide participates in the formation of the degenerative structures listed above is based on the temporal and spatial relationship of the metachromatic material to

these structures and to the fact that the latter substances give a positive polysaccharide aldehyde reaction. In the early stages of fibrinoid formation, for example, accumulation of acid mucopolysaccharide in the connective tissue usually is observed (mucinous edema of Talalajew [29]). Similarly, an increase in metachromasia [30] or loosening of the ground substance [31] is recognized as an early phase of the sclerotic process. The positive polysaccharide reaction of the degenerative structures has been noted previously [32] and in the present study. The chemical and physical properties of the acid mucopolysaccharides are such that their participation in the formation of the degenerative structures is entirely possible. Morphologic and experimental data bearing on this point will be discussed more fully below.

(g) Acid mucopolysaccharide formation may occur with or without cellular proliferation. The association of growing tissues and metachromatic substances is noted in neoplasms, embryos, lesions produced by desoxycorticosterone or lyophilized anterior pituitary extract, granulation tissue, and in the tissue alterations of scorbutus. This relationship also has been noted previously.[33,34] Chromotropic substances, however, may form also when there is relatively little cellular proliferation. This occurs, for example, in the hearts of patients with disseminated lupus erythematosus [35] and rheumatic fever,[1] in the renal medullary substrate, and in arteriosclerotic vessels. This finding is of interest since Rössle's [6] concept of sclerosis is that of hardening and induration of connective tissue which has formed without previous granulation, *i.e.*, without significant fibroplasia and angioplasia (*vide infra*).

Review of Factors That Affect Serous Inflammation

Serous inflammation has been the object of much physiologic and chemical study. Selye [36] recently has referred to many of the early findings in his articles on the general adaptation syndrome, and Rössle,[6] Eppinger and co-workers [3,4] have written extensively on the subject. An exhaustive presentation, therefore, will not be attempted, but the pertinent conditioning factors will be considered briefly.

Capillary Permeability. The factors that influence the passage of fluid from the blood stream into diseased tissues are complex.[37] For example, it has been demonstrated that capillaries from various anatomical sites differ in their membrane characteristics,[38] and factors such as blood flow, vasomotion, loss of integrity of the endothelial linings, osmotic properties of the serum, and alterations of the tissue colloids complicate the interpretation of the results of technics designed to measure primary alterations of capillary permeability. Nevertheless, increased passage of fluid and large-molecular dyes from the circula-

tion into tissues has been noted in a variety of lesions falling within the scope of serous inflammation. Eppinger [3,4] noted this increased permeability in his experimental animals; Lange [39] observed the phenomena in myxedema; Rigdon and co-workers [40,41] noted the change after exposure to x-radiation and tissue extracts; and Hechter [42,43] reported identical findings after the administration of estrogens and histamine. Whether these alterations are primary or secondary, however, is not known.

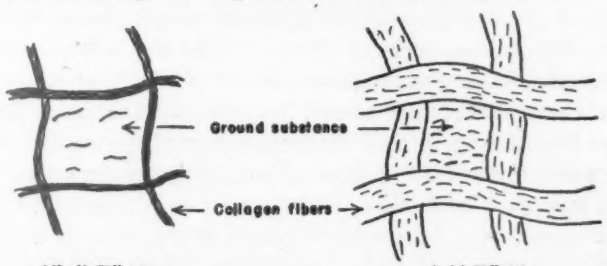
TABLE I *

The Influence of Certain Factors on Collagen and Ground Substance

Effect of:	Ground substance	Collagen
Hydrogen ions	Slight swelling	Much swelling
Hydroxyl ions	Much swelling	Slight swelling
Distilled water	Much swelling	Shrinkage and coagulation
Dilute salt solutions	Much swelling	Slight swelling
Concentrated salt solutions	Shrinkage	Swelling

* Modified from Schade.[2]

Tissue Permeation. Schade [2] believed that the extracellular connective tissue elements were importantly involved in tissue permeation and in the regulation of the acid-base, water-electrolyte, and osmotic equilibria. The essential features of his view are that the extracellular elements—the amorphous ground substance and the fibers—function as a two-colloid system and that the properties of the constituent colloids make them eminently suitable for action as a body regulator. Text-Figure 2 and Table I indicate the general properties of the constituents. It will be noted that the colloidal reactions are antagonistic in many respects, and thereby the "regulator" action is enhanced.



Text-Figure 2. The effect of acid and alkali on connective tissue elements. Modified from Schade.[2]

In addition to the tabulated factors, specific ions are of some influence in determining the colloidal state of the connective tissue. For example, increase in the sodium ion is accompanied by increased dispersion of the colloids of the ground substance and water retention, whereas an accumulation of potassium and calcium is followed by shrinkage and loss of fluid.

The characteristic movements of water and electrolytes in serous inflammation tend toward dispersion of the colloid and decreased viscosity. Indeed, Ragan and Meyer [44] reported increased quantities of acid mucopolysaccharide but decreased viscosity in the synovial fluids from patients with rheumatoid arthritis.

Schade [2] supposed that forces which cause dispersion of the ground substance cause increased diffusibility, whereas gelating forces have an opposite effect. However, contrary to this supposition, it has been found that increased tissue hydration, as occurs in serous inflammation, is accompanied by decreased spread in the tissues.[45,46] Explanation of this phenomenon is not certain, but it is known that other factors such as capillary forces, electroviscous effects, tissue tension, mechanical massage, and adsorbed substances are of significance in tissue permeation.[45]

In recent years an important specific factor in the spread of particulate substances has been discovered. The researches of Duran-Reynals,[45] Meyer,[47] Chain and Duthie,[48] Clark and Clark,[49] S. Bensley,[50] McClean,[51] and others have resulted in the identification of the "spreading factor" with hyaluronidase, an enzyme complex capable of depolymerizing and hydrolyzing hyaluronic acid. This latter substance is one of the group of hydrophilic acid mucopolysaccharides and is an important constituent of the ground substance. Thus, the action of the spreading factor is believed to be due to depolymerization of the viscous ground substance and a lowering of this mechanical barrier. In spite of considerable work, however, the significance of the spreading factor in physiologic and pathologic processes is unknown. For this reason, the importance of the serologic and pharmacologic anti-hyaluronidases is undecided. The biology and chemistry of these and related substances have been the object of numerous reviews.[45,47,52-54]

In summary, then, it may be said that, although the connective tissue plays an integral rôle in the hematoparenchymal system, it is still not certain whether it is the limiting factor or the primarily altered constituent in any given instance.

Cell Permeability. Measurement of cell permeability is particularly difficult in tissues, and theories based on primary alterations in this sphere are almost all purely inferential. Alterations in electrolyte content of diseased tissues, for example, are frequently taken to indicate changes in cell permeability and, indeed, these changes do occur in serous inflammation. However, it cannot be stated with certainty whether a given change is due to primary alteration of permeability, of cell metabolism, or of extracellular forces.[38,55]

Electrolytic Alterations. The essential features of the acute electrolytic alterations in instances of serous inflammation have been de-

scribed by Kaunitz.[56] They consist of a loss of tissue potassium and phosphate, and a gain in sodium, chloride, and water. The nature of the methods utilized to induce these changes was quite general and included suffocation, exposure to diphtheria toxin or to allyl formate, and hypersensitivity reactions. Kaunitz also carried out studies on electrolyte balance, with compatible results, on patients with hypertension and angioneurotic edema. Findings consistent with this "transmineralisation" have been reported also in histamine and anaphylactic shock; after the administration of desoxycorticosterone acetate or estrogens, and after exposure to a variety of other damaging agents.

Metabolic Alterations. The major metabolic alteration consists of increased glycolysis (breakdown of carbohydrate with the formation of lactic acid).[8,28,57,58] The associated protein changes vary. In some instances there may be increased protein catabolism [59] or decreased protein synthesis,[60] and in other cases increased protein formation occurs. In some instances these changes are correlated with a decreased tissue content of respiratory enzymes [61] and, at times, other enzymatic abnormalities (*e.g.*, decreased oxidative deamination) have been recorded.

The exact manner in which these changes are induced is not known, but it is apparent from clinical and experimental evidence that concomitant electrolytic, nutritional, and endocrine alterations and the disturbed physical characteristics of the tissue are of importance. For example, serous inflammatory lesions may be potentiated by diets rich in sodium and may be ameliorated somewhat by diets low in this ion.[36,62] Similarly, deficiencies in vitamins B_1,[3,4,6] C,[4,63] and probably of E [64] will produce or potentiate this alterative process.

The hormonal aspects of the problem have been and are being studied widely. Current preoccupation with the pituitary-adrenal function, both from etiologic [36] and therapeutic [65,66] viewpoints, is evidence of this interest. Previous observations and the present investigation indicate that the hemato-parenchymal system is conditioned also by estrogen, androgen, progesterone, thyroid, and insulin metabolism.[3,4,45]

Rôle of Acid Mucopolysaccharide in Degenerative Processes

Numerous studies dealing with the pathogenesis of sclerotic, amyloid, hyaline, and fibrinoid material have indicated the basic similarities in their development and the importance of the reactivity of the ground substance. It is not possible, in this presentation, to refer to the voluminous literature bearing on this aspect. Excellent bibliographies are found in articles by Volland,[67] Heinlein,[68] Linzbach,[69,70] and Bredt.[71] In summary, features common to the formation of these degenerative structures are: (a) occurrence of extracellular connective tissue,

(b) edematous (mucinous) bed, (c) similar staining reactions, (d) similar morphologic appearance, (e) development by means of similar damaging agents, and (f) transition of one form into another.

To clarify the discussion, a short description of the various degenerative structures is offered.

Fibrinoid. Fibrinoid formation refers to the appearance in the connective tissue of a homogeneous, eosinophilic, refractile, relatively acellular, band-like structure which has some of the tinctorial properties of fibrin. Fibrinoid has been described in cysts, bursae, and ganglia; in the base of peptic ulcers; in lesions associated with the Arthus phenomena; in rheumatoid arthritis; rheumatic fever; disseminated lupus erythematosus; malignant hypertension; placenta; arteriosclerosis; and in the "hyaline membranes" of the lung.[72] (For bibliography, see reference 1.) Fibrinoid alteration, contrary to hyalinization or sclerosis, is believed to be a temporary stage in a complex inflammatory process. Bredt,[71] for example, believed that fibrinoid formation may go on to sclerosis or hyalinization, and other authors have considered it to be related to amyloid development.[67,73]

Sclerosis. As indicated above, Rössle [6] defined sclerosis as an induration of the connective tissue with the development of a homogeneous, refractile, acellular, unabsorbable, presumably non-functioning deposit with staining properties characteristic of connective tissue. The formation of this material is believed to occur without previous granulation. This process is said to be the end stage of serous inflammation.

Connective Tissue Hyalin. In later stages it is not possible to differentiate sclerosis from connective tissue hyalinization. In the early phases, however, hyalin is believed to be associated with granulation. The relation of connective tissue hyalinization to amyloid formation has been stressed.[74-76]

Amyloid is an amorphous, hyalin-like material which is distinguished by its characteristic distribution and its staining reactions. Amyloid is commonly divided into four groups: the secondary form associated with chronic disease; the local or tumor variety; the systemic form associated with multiple myeloma; and the primary systemic type. The secondary form of amyloid is characteristically extra-endothelial and involves the spleen, liver, kidney, and adrenal. The other varieties may affect connective tissues anywhere in the body and generally do not involve the structures affected in the secondary type. The secondary form shows no chromotropism with toluidine blue, whereas the other types of amyloid lie in a metachromatic bed.[77] Secondary amyloid, however, does give a positive polysaccharide aldehyde reaction with pe-

riodic acid leukofuchsin, and Hass'[78] chemical analysis indicates that acid mucopolysaccharides are present.

The Formation of the Degenerative Lesions

Histologic and histochemical evidence, then, suggests that the metachromatic material participates in the formation of the degenerative lesions referred to above. This, of course, is not a new idea. Klinge[79] and Bahrmann,[80] for example, referred to the rôle that the ground substance played in fibrinoid formation; Aschoff,[81] Hueck,[81] and others were concerned with metachromatic substances in the pathogenesis of arteriosclerosis; Volland[67] and Knežević[73] implicated these substances in amyloid or paramyloid formation.

Although the exact mechanism by which these pathologic changes occur is not known, the chemical and physical properties of acid mucopolysaccharide (AMP) suggest certain possible reactions, some of which follow:

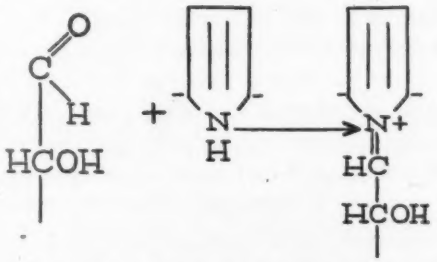
(a) Protein reacting as a base + AMP → protein–AMP complex.[82]

(b) Complex protein + AMP → protein–AMP complex + liberated group, *e.g.*, cephalin–protein + AMP → protein–AMP complex + cephalin.[83,84]

(c) Hydrophilic colloid + AMP → hydrophilic colloid + AMP.[85]
(dispersed) (flocculent)

(d) (colloid) + (AMP) → (colloid–AMP).[86,87]
(coacervate)

(e) Carbohydrate + primary or secondary amine → nitrogen glycoside.[88] *e.g.*:



The dependence of these colloidal reactions on physicochemical conditions (the ionic strength and pH of the medium and the degree of dispersion or polymerization of the reactants) is well recognized.

In a previous communication,[1] the opinion was expressed that fibrinoid probably was formed by the co-precipitation of an alkaline protein and the acid mucopolysaccharide of the ground substance (reaction "a"

above). The bases for this belief are: (a) the temporal, spatial, and configurational relationship of fibrinoid and metachromatic substances; (b) the positive polysaccharide and arginine reactions of fibrinoid; (c) the increase in tissue pH and the liberation of alkaline substances in lesions generally associated with fibrinoid formation; (d) the failure to obtain consistently positive fibrin (phosphotungstic acid hematoxylin), lipoidal, or Feulgen reactions in the fibrinoid material; and (e) the occurrence of fibrinoid or fibrinoid-like structures in areas where necrosis of muscle or of collagenic, reticular, or elastic fibers cannot occur or where its occurrence is unlikely. This opinion, of course, can be conclusively established only by the isolation and chemical analysis of the fibrinoid material from various anatomical sites.

Since cholesterol may be stabilized by lipoprotein, it has been suggested that reaction "b" may be of importance in its deposition in chronically inflamed tissue and in the arteriosclerotic aorta.[89]

An interpretative analysis of the tissue reactions supports the contentions of Eppinger [3,4] and Rössle [6] that the pathogenesis of certain degenerative diseases of blood vessels and connective tissue lies within the scope of serous inflammation. The value of this concept lies in its correlation of various clinical, pathologic, and therapeutic phenomena, and its aid in the interpretation of experimental data. For example, suggested therapeutic measures have sought to correct isolated abnormalities observed in serous inflammation. The low sodium, vegetarian diets of Kaunitz [56] and Kempner,[62] for example, are directed toward the electrolyte abnormalities; testosterone,[61] the correction of the negative nitrogen balance; anti-reticular cytotoxic sera,[90] the stimulation of connective tissue functions; and succinate,[91] cytochrome,[92] and adenosine triphosphoric acid [93] have been utilized to correct the depressed respiration.

The non-specific character of the tissue reactions indicates the caution with which one must proceed in ascribing etiologic significance to a particular agent. Although it may be possible to produce highly suggestive lesions by means of a damaging mechanism, etiology is indecisive until *that mechanism* is shown to be operative in a significant number of clinical instances of the disease in question. These precautions should apply also to the interpretation of the mode of action of a given therapeutic agent. A substance may correct an isolated abnormality, for example, and alleviate symptomatology without necessarily affecting the basic dysfunction.

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ARTERITIS OF STRIATED MUSCLE IN RHEUMATOID ARTHRITIS*

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A study of striated muscle from 57 cases of rheumatoid arthritis has disclosed inflammatory changes of arteries in 5 instances. The tissue examined was obtained from muscles that were not situated near joints involved by the arthritic process. The vascular lesion cannot, therefore, be related directly to, or considered to be extensions of, the inflammatory changes in the joints. Furthermore, vascular lesions were not observed in 111 specimens of striated muscle from conditions other than rheumatoid arthritis that were studied histologically by the same procedures. The arteritic lesion must therefore be considered characteristic of rheumatoid arthritis even though its incidence is low, 8.8 per cent. The control group of non-rheumatoid arthritis consisted of 55 patients with other forms of arthritis, 6 with subacute bacterial endocarditis, and 50 with miscellaneous conditions.

Among other groups of 21 persons with active rheumatic fever and 11 with inactive rheumatic heart disease, only one specimen of striated muscle contained a single acutely inflamed artery. This was from a case that was regarded clinically as inactive rheumatic heart disease. In one of 2 specimens of muscle from patients with disseminated lupus erythematosus, very mild arteritic lesions were present. The character of the inflammatory reaction in these two specimens differed in many respects from that observed in the rheumatoid arthritis group. Both rheumatic fever and lupus erythematosus are diseases in which disseminated vascular lesions are known to occur. The finding of arteritis in these 2 cases is therefore understandable.

The average volume of muscle studied from each of these 202 cases was estimated to be 0.32 cc. On the average 400 serial sections, 8 μ in thickness, were prepared from each case and every fifth section was examined histologically. In the 5 cases of rheumatoid arthritis with arteritis, only a few sections revealed lesions. It is apparent, therefore, that the usual method of examining a few random sections of muscle fails to disclose the presence of this lesion in most instances. Nevertheless, a sixth example of arteritis was found in routine sections from a case of

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rheumatoid arthritis not included in the 57 that were studied by serial section.

Baggenstoss and Rosenberg [1] paid particular attention to the visceral blood vessels in necropsies of patients with rheumatoid arthritis but failed to note significant changes. In one of Rich's tables,[2] arteritis is listed as a mild or infrequent lesion of rheumatoid arthritis. Graef, Hickey, and Altmann [3] described inflammatory changes in the coronary arteries of 2 cases of this disease. Ellman and Ball [4] noted inflammatory changes in blood vessels of the heart, lungs, and kidneys in one case of rheumatoid arthritis.

Vascular lesions of striated muscle do not appear to have been described previously in rheumatoid arthritis. The lesion is of interest because it furnishes one more point of similarity to the several that have been recognized between rheumatoid arthritis and rheumatic fever. The subcutaneous nodules in both may be strikingly similar histologically.[5] In addition, cardiac lesions simulating, if not identical with, those of rheumatic fever have been described at necropsy in many cases of rheumatoid arthritis. The occurrence of arteritis in a small percentage of cases of rheumatic fever,[6] albeit not usually in striated muscle, is well recognized. The arteritis of rheumatoid arthritis may have much the same basis as the arteritis of the rheumatic state.

It is of interest also that arteritic lesions have been recognized in a number of conditions in which allergic phenomena are prominent and in those in which destruction of collagen is a conspicuous feature of the lesions. These include periarteritis nodosa, rheumatic fever,[6] serum sickness,[7] and lupus erythematosus.[8] Rheumatoid arthritis has sometimes been included in these groups of diseases.

It is the purpose of this report to describe the vascular lesion encountered in these cases of rheumatoid arthritis in some detail and to compare it with other varieties of arteritis.

Report of Cases

Case 1

C. B., a white female, 64 years old, was admitted for pain, redness and swelling of ankles, knees, shoulders, and hands of 7 months' duration. This was her initial attack. She had lost 28 lbs. during the past 3 years, of which 15 were lost in the past 7 months. Although not bedridden, she walked with difficulty. Physical examination showed fusiform swelling of the interphalangeal joints with loss of flexion and extension, slight ulnar deviation, limitation of motion of both shoulders, and swelling of wrists. Examination of the heart, lungs, and neurologic function were negative. There were no subcutaneous nodules or skin lesions. The temperature was 100.6° F.; pulse, 96; respirations, 20; blood pressure, 130/74 mm. of Hg. The erythrocyte sedimentation rate was 104 mm. (Westergren). The clinical diagnosis was rheumatoid arthritis. During the next 9 months in a hospital for chronic diseases, there was

a progression of joint involvement, intermittent periods of fever, and the development of bone and joint changes that were considered by roentgenologic examination to be typical of rheumatoid arthritis.

Biopsy of the gastrocnemius muscle (no. 2855/47): One medium-sized artery had a fairly marked infiltration of the adventitia and of the external portion of the media by polymorphonuclear neutrophils and plasma cells. At one pole of the artery this infiltration extended through the entire thickness of the media and involved the subintimal layer. The arterial wall was thickened and the lumen narrowed. The thickening was caused partly by widening of the media by edema and partly by intimal proliferation. Another artery of the same size was thickened and narrowed by intramural cellular infiltration. The adventitia of this vessel contained a moderate focal infiltration of large mononuclear cells, polymorphonuclear neutrophils, and plasma cells. The endothelium of both vessels was intact and the lumina patent. No additional changes were noted in Weigert's elastic tissue stains other than some fraying and reduplication of the internal elastic lamella in the second of the two vessels. The muscle fibers throughout were well preserved.

Case 2

S. S., a white woman, 63 years of age, developed pain and swelling of the left knee 6 months prior to admission and pain without swelling of hands some time later. Several injections of a gold salt were given without benefit or toxicity. There was a marked sense of fatigue and a loss of 40 lbs. in weight. On admission the knees, ankles, and proximal interphalangeal joints were painful, tender, stiff, swollen, and warm. There was some flexion deformity of the knees. Roentgenograms revealed changes of rheumatoid arthritis in the interphalangeal joints of hands and feet and in the wrists with marked osteoporosis. Red blood cells were 3.37 millions per cmm.; hemoglobin, 10.3 gm.; white blood cells, 6,300 per cmm. with 97 per cent polymorphonuclear neutrophils. Erythrocyte sedimentation rate was 65 mm. (Westergren); agglutination test for hemolytic streptococci was strongly positive; the serum calcium was 10.0 mg. per cent; serum phosphorus, 3.85 mg. per cent; uric acid, 2.8 mg. per cent.

Biopsy of gastrocnemius muscle (M175): Four hundred and four sections were cut serially and 80 stained and examined. Inflammatory changes were noted in two small arteries measuring 225 and 170 μ in their largest external diameters. In the larger vessel the adventitia was thickened and consisted of several concentric thin layers of collagen between which were interspersed large mononuclear cells, lymphocytes, and a few cells with irregular pyknotic nuclei. The media and internal elastic lamella were intact. The intima was moderately and concentrically thickened by edematous connective tissue in which a few mononuclear cells were scattered. The endothelium was intact. The process extended along several smaller branches of this vessel for

a considerable distance. Around one such branch was a localized infiltration of plasma cells, lymphocytes, and mononuclear cells with both vesicular and dense nuclei. A few of the infiltrating cells were polymorphonuclear leukocytes. The infiltration had the form of a crescent extending for about one-half the circumference of the vessel. The muscle coat of this vessel was hypertrophied but otherwise unaltered. The lumen was patent but narrow. In the second lesion a small thick-walled artery was encircled by a thin rim of cells with pyknotic nuclei, lymphocytes, and a few larger mononuclear cells. The smooth muscle cells of the thick media were disarranged along one side and fibroblasts were intermingled with them. The elastica was beaded and showed exaggerated and redundant undulations as though the vessel were in a markedly contracted state. The lumen was reduced to a minute eccentrically placed aperture with an intact but swollen endothelium. Marked overgrowth of intimal fibrous tissue was noted and this newly formed fibrous tissue contained scattered leukocytes. Necrosis of collagen or "fibrinoid" change was not noted.

The muscle tissue itself was well preserved but at scattered points there were seen several focal collections of lymphocytes such as have been described in rheumatoid arthritis [9] and other conditions.[10]

Case 3

D. S., a Negro male, 32 years old, was admitted with pain and swelling of the hands of 6 months' duration. This had been gradual in onset, beginning in the left wrist and fingers and later involving in succession, the right hand, left ankle, knees, right ankle, toes, and left shoulder. He had had a previous mild attack involving the fingers 7 years ago; it lasted 18 months and subsided spontaneously. There had been a weight loss of 20 lbs. He had had gonorrhea 12 years previously. The involved joints were painful and stiff. Roentgenograms revealed changes consistent with rheumatoid arthritis. He had a low-grade fever; red blood cells, 3.37 millions per cmm.; hemoglobin, 13.2 gm.; white blood cells, 6,100 per cmm.; polymorphonuclear neutrophils, 69 per cent; erythrocyte sedimentation rate, 94 to 134 mm. (Westergren); uric acid, 2.7 mg. per cent; agglutination test for hemolytic streptococci, positive; Wassermann test of the blood, negative; albumin/globulin ratio, 3.8 gm./3.0 gm.; prostatic smear negative for Gram-negative diplococci. He never had received gold therapy. Clinical diagnosis: rheumatoid arthritis.

Biopsy of gastrocnemius (M127): Six hundred and three sections were cut and 120 stained and examined. One small artery, 210 μ in external diameter, cut longitudinally, contained a sharply localized area of acute inflammation that involved all three layers of the vessel wall. An infiltration of leukocytes extended between collagen fibers of the adventitia and penetrated through the muscle coat, disrupting the continuity of smooth muscle cells. The infiltrating cells had irregular elongated and distorted nuclei and were difficult to identify. A fair proportion appeared to be polymorphonuclear neutrophils, but lympho-

cytes and large mononuclear cells also were present. The endothelium was intact but swollen, and lifted from the basement membrane by a coagulum of protein and agglutinated erythrocytes. No thrombus was superimposed. There was some fibroblastic and endothelial cell proliferation. In serial sections this lesion was seen to extend for some distance in the wall of the vessel and to involve one branch. At one point there was an irregular cleft that entirely disrupted the media and broke through the internal elastic lamella. Elsewhere the elastica was still intact. At another point a newly formed capillary extended into the media from the adventitia. In the involved branch the inflammatory reaction was largely adventitial, a narrow rim of various leukocytes forming a ring around the vessel. The intima of this vessel, however, was slightly swollen and edematous. The striated muscle throughout all sections was unchanged and no lymphocytic accumulations were found.

Case 4

T. K. was a white female, 50 years of age, whose first attack of arthritis occurred 6 years before, lasted 8 months, and involved the neck and left knee. The following year arthritis returned and gradually spread to involve almost all joints with deformity and stiffness. She had been confined to bed for 4 years. Treatment at various times had included typhoid vaccine, milk, gold and liver injections, as well as physiotherapy. She had not had rheumatic fever or gonorrhea. On physical examination, stiffness and deformity of the fingers, wrists, elbows, shoulders, toes, and knees were marked; in addition there was swelling, pain, and tenderness of fingers and the right shoulder. The erythrocyte count varied from 4 to 5 millions per cmm. The white blood cell count was normal. The Wassermann reaction of the blood was negative. The erythrocyte sedimentation rate varied from 34 to 66 mm. (Wintrobe). Roentgenologic examination revealed advanced changes of rheumatoid arthritis in joints and skeleton. The agglutination test for hemolytic streptococci was positive.

Biopsy of gastrocnemius muscle (M62): Two hundred and six sections were cut and 40 stained and examined. Lesions of varying extent and in different stages of development were noted in 8 vessels at scattered points. Most of these were very small arteries or large arterioles about 100 μ in diameter. One larger artery, 420 μ in diameter, revealed an arrested lesion. Irregular areas of the media were scarred and the elastica was extremely redundant and contained several small breaks. The intima was cellular and concentrically thickened. A second small artery, 185 μ in diameter, cut obliquely, had a crescentic nodule of leukocytes that formed a cap at one margin and extended into the external aspects of the media. The leukocytes were of several types, with pyknotic nuclei predominating. The elastica beneath this area was thinned out to the point of complete dissolution and the subjacent intima was thickened. In special stains both collagenous and reticulum fibers were found to course through the inflammatory focus. In a third artery of the same size, the acute process was pronounced and involved

all three layers, so that only small remnants of the original vessel wall could be detected even with elastic tissue stains. The lumen was obliterated by the intramural process rather than by thrombosis. The very small arteries or arterioles showed only mild acute inflammatory changes. The muscle fibers throughout the specimen were considerably atrophied and there were numerous small, compact, miliary or submiliary aggregates of lymphocytes. These latter lesions were independent of the vascular lesions in distribution.

Case 5

I. T. was a white male, 36 years old, whose arthritis had been initiated by an attack of acute pericarditis 2 years previously. Subsequently he developed joint pains and subcutaneous nodules. Examination of subcutaneous tissue and muscle by biopsy at that time revealed inflammatory changes that suggested "dermatomyositis," but this diagnosis was never established. Two months previously he had been admitted with fever and limitation of motion of elbows, fingers, and knees. He developed subcutaneous nodules on the ulnar aspect of the arms, one of which was excised and reported as a "non-specific nodule of the rheumatoid arthritis type." Laboratory data were within normal limits except for a 3 plus cephalin flocculation test and a 2 plus thymol turbidity test. No objective joint changes were noted on physical examination and roentgenograms of hands, knees, and spine were normal. He developed pneumonia in the hospital and was treated with penicillin. He became afebrile. An electrocardiogram was normal and there were no cardiac abnormalities. Erythrocyte sedimentation rate was 32 mm. (Wintrobe). Agglutination test for hemolytic streptococci was doubtful. Another subcutaneous nodule again revealed changes typical of rheumatoid arthritis.

Biopsy of gastrocnemius muscle (M182): Four hundred sections were cut and 80 stained and examined. Four vessels at scattered points were acutely inflamed. The largest of these measured 120 μ in external diameter; the smallest was an arteriole about 75 μ across. In all, the adventitia was invaded by dense collections of leukocytes, among which polymorphonuclear leukocytes predominated but in which lymphocytes and large mononuclear cells were fairly abundant. In three vessels the entire circumference of the vessel was involved, but in the fourth the adventitial collection of leukocytes was limited to one pole. The media of the vessels was invaded and partly obliterated by leukocytes, but with elastic tissue stains the elastic lamellae were remarkably well preserved and intact. The intimal layer was thick and cellular, and the lumen greatly encroached upon but not totally obstructed or thrombosed.

The striated muscle showed many scattered areas of atrophy of the fibers but there was no evidence of a diffuse myositis. However, a number of characteristic lymphoid aggregates were found in the endomysium, perimysium, and epimysium.

Case 6

F. B. was a white male, 53 years of age, who developed generalized swollen and painful joints several weeks before the specimen was taken for biopsy. He had had a previous episode of widespread polyarthritis 11 years previously with complete remission after 9 months. On physical examination there was stiffness, pain, tenderness, and swelling of fingers, wrists, elbows, toes, and knees. The temperature ranged from 99° to 104° F. Erythrocyte count was 4.08 to 5.19 millions per cmm.; hemoglobin, 11.0 gm.; erythrocyte sedimentation rate, 34 mm. (Wintrobe); Wassermann test of the blood was negative. The patient had lost 18 lbs. during the present illness.

Biopsy (M16) of quadriceps femoris muscle: Six hundred sections were cut and 120 stained and examined. A single, small, relatively thin-walled artery cut in a slanting plane contained a small intramural granulomatous lesion about the size of a miliary tubercle at one pole. The vessel measured approximately 175 μ in diameter and the lesion, 95 μ. The latter consisted of conglomerated leukocytes, among which polymorphonuclear cells were the most conspicuous. The nodule protruded into both the lumen and the adventitia but centered on the media of the vessel, totally obscuring the smooth muscle layer at that point. In elastic tissue stains remnants of the elastic lamella were found within the cellular focus and there was splitting and fraying of elastic tissue beyond this zone. The remainder of the arterial wall was unchanged. No other vessel was involved. The striated muscle was unaltered. No lymphocytic aggregates were found.

Resumé of Arteritic Lesions

Analysis of the lesions in these 6 cases discloses that there are no very distinctive features that sharply differentiate this form of arteritis from other recognized varieties. Nevertheless, there is an obvious uniformity and consistency in the major features. The adventitia regularly shows the most pronounced change and in some instances is the only coat appreciably involved. However, often the inflammatory changes have spread through all three layers and the lesion may therefore be termed panarteritis.

The cellular exudate is varied in composition but the usual types of cells found in granulomatous lesions are encountered. Polymorphonuclear neutrophils predominate in some instances while in others, presumably older lesions, mononuclear forms are more conspicuous. A large proportion of the infiltrating cells have distorted, pyknotic nuclei and cannot be accurately identified. Proliferation of endothelial cells and fibroblasts regularly accompany the cellular infiltration but to a variable extent. Peculiar or distinctive varieties of cells such as eosino-

phils, multinuclear giant cells, or large basophilic cells such as are found in Aschoff nodules are not encountered.

There is no evidence that the initial event is destruction of some component of the vascular wall and that the cellular reaction is a secondary phenomenon. In more severe lesions there is destruction of smooth muscle and elastic tissue but this is seldom prominent and usually appears to be the result of the inflammation and not to have preceded it. The integrity of the endothelial lining is seldom lost although the endothelial cells often are swollen and arranged in several concentric layers. Consequently, as in rheumatic arteritis, thrombosis seldom occurs. In many instances the muscular wall appears to be unusually thick and the undulations of the internal elastic lamella are especially prominent as though the vessel were in a state of spasm during the development of the lesion. Necrosis of collagen with intensification of acidophilic staining of collagen, such as is prominent in the lesions of periarteritis nodosa and rheumatic arteritis, is not a distinctive feature of the arteritic lesion of rheumatoid arthritis.

There is a fair degree of uniformity in the size of the vessels that are injured. These vary from 75 to 420 μ in diameter or, in other words, from large arterioles to small arteries. Small arterioles and larger muscular arteries are spared.

Although lesions were found in cases of rheumatoid arthritis that had persisted over many years, there is little evidence that the vascular lesions were of equal duration or that they had progressed by a series of recrudescences. Progressive lesions of this nature frequently are noted in the arteritis of rheumatic fever, in periarteritis nodosa, and in thromboangiitis obliterans. In most instances, however, the lesions of rheumatoid arteritis could readily be classified as acute or subacute in nature rather than as chronic. In only a single vessel (case 4) did there appear to be residual scarring from previous active inflammation. There is no reason to believe that active inflammatory changes should be present only at the time of biopsy. It may be presumed, therefore, that these inflammatory lesions seemingly heal by resolution without appreciable permanent injury.

Discussion

From the foregoing analysis it is evident that the specificity of the arteritic lesion in striated muscle in rheumatoid arthritis depends chiefly upon the location of the lesion, the size of the artery involved, and the association with joint or subcutaneous lesions rather than upon any distinctive quality of the inflammatory process itself. The differences from other varieties of arteritis are largely quantitative rather than

TABLE I

Comparison of the Chief Characteristics of Several Types of Arteritis

	Rheumatoid arthritis	Rheumatic fever	Periarteritis nodosa	Lupus erythematosus	Giant cell arteritis	Thromboangiitis obliterans
Incidence of arterial involvement	8.8%	Less than 5%	Constant	Common	Constant	Constant
Commonest site	Striated muscle	Lungs	Splanchnic area	Kidney	Temporal vessels	Lower extremities
Size of artery	Small muscular	Large and small muscular	Variable	Small and medium muscular	Arterioles and small arteries	Large and some small muscular
Layers of vessel wall chiefly involved	Adventitia	Intima and media	Panarteritis	Intima and media	Media	Panarteritis
Predominant or characteristic cells of exudate	Large mononuclear and polymorphonuclear	Large mononuclear and polymorphonuclear	Eosinophil and large mononuclear	Proliferating endothelium	Multinuclear giant cell	Variable
Necrosis or degeneration of collagen	Slight	Moderate	Marked	Marked	Slight	Not prominent
Endothelial cell injury	Slight	Slight	Marked	Marked	Slight	Marked
Involvement of elastica	Secondary	Secondary	Marked	Not described	Marked, probably primary	Marked
Thrombosis	None	None	Common	Common	Fairly common	Common
Intramural vascularization	Slight	Marked	Moderate	Not described	Slight	Marked
Aneurysm formation	None	None	Common	None	None	None
Lesions in multiple stages of development	Occasionally	Common	Common	Common	Common	Very common
Commonest associated cardiac lesions	Pericarditis	Myocarditis and endocarditis	None	Atypical verrucous endocarditis	None	None

qualitative. Some of these differences have already been mentioned; others are brought out in Table I, where a comparison of the important features of arteritis of several common types is listed.

The arteritis of rheumatoid arthritis resembles that of rheumatic fever in incidence and in the absence of marked endothelial cell destruction and thrombosis. Moreover, in both of these diseases cardiac lesions are recognized. Rheumatoid arteritis differs from rheumatic arteritis in location of the lesion, in the range in size of arteries involved, in the lack of conspicuous collagen damage and intramural vascularization. Finally, polymorphonuclear leukocytes are a more conspicuous feature of the inflammatory exudate of rheumatoid arteritis than of the rheumatic lesion.

Rheumatoid arteritis resembles the lesion described in human serum sickness [7] in the character of the inflammatory exudate produced, but, in the latter, intimal involvement is striking and mural endocardial involvement prominent. Rheumatoid arteritis is unlike periarteritis nodosa in several significant respects, namely, in distribution, in size of vessels involved, and in lack of eosinophilia, collagen necrosis, aneurysm formation, and thrombosis. The vascular lesion of lupus erythematosus occurs more commonly in arterioles than in arteries and may also involve veins. Venous involvement was not noted in any of the cases of rheumatoid arthritis. Furthermore, the lesion of lupus erythematosus is more often a degenerative rather than an inflammatory one. The differences between rheumatoid arteritis and both giant cell arteritis and thromboangiitis obliterans are even more impressive (Table I) than between rheumatoid arteritis and the other varieties of arteritis.

The clinical and pathologic data in these 6 cases are sufficiently diverse to indicate that the vascular lesion is not readily associated with any particular feature of rheumatoid arthritis. For example, it cannot be related to the severity or duration of arthritis since these are variable, and in one case, no. 5, only slight joint involvement was present. The following features were lacking in one or more cases: (1) therapy with gold compounds or vaccines, (2) agglutinins for hemolytic streptococci, (3) subcutaneous nodules, (4) marked elevation of erythrocyte sedimentation rate, (5) interstitial foci of lymphocytic infiltration in muscle, (6) diffuse muscle atrophy or other muscular abnormality. The development of arteritis cannot, therefore, be related to these inconstant features of rheumatoid arthritis.

The cause of rheumatoid arteritis is as obscure as is that of the other varieties of non-infectious arteritis. The tendency for most of these processes to be limited to small muscular arteries and to spare large

arteries and veins is a striking phenomenon, for which there is no adequate explanation. The tissue structure, and presumably composition, of small arteries does not vary sufficiently from that of other vessels to suggest that they may be injurious agents that act selectively on only this segment of the vascular system. It is more logical to suppose that the dynamics of circulation are such as to permit the entrance and accumulation of toxic substances in higher concentration in small muscular arteries than elsewhere.

The most conspicuous detail in which the small arteries differ from other vessels in relation to the circulation is that in them the systolic pressure is almost as great as in the large arterial trunks, yet their vessel walls are relatively thin. In other words, they represent the portion of the vascular system in which the ratio of blood pressure to vessel wall thickness is far greater than in any other portion with the possible exception of the capillary bed. It is likely that the inciting agent that provokes the reaction of arteritis in rheumatoid arthritis and other forms of vascular disease penetrates into the wall through the endothelium from the circulating blood. It is probable that these noxious substances are of such molecular magnitude that they can penetrate endothelium only at points of relatively high intravascular tension and can reach the adventitia only in vessels that are relatively thin.

Summary

Arteritis was found in 5 specimens of striated muscle, studied in serial sections, from 57 cases of rheumatoid arthritis, and in casual sections of striated muscle from a sixth case of rheumatoid arthritis. Although not possessing distinctive histologic characteristics, the lesion is believed to be a specific manifestation of the disease because (1) it does not occur in striated muscle from conditions other than rheumatoid arthritis, (2) the histologic features of the inflammatory reaction are fairly constant, and (3) the process is limited to very small arteries.

The arteritis of rheumatoid arthritis resembles non-infectious arteritis of other types in some respects and differs in others. Necrosis of collagen and intramural vascularization do not appear to be important manifestations. The lesion otherwise resembles the arteritis seen in association with rheumatic fever. It is suggested that the arteritis of both rheumatoid arthritis and rheumatic fever may have analogous relationships to the underlying disease processes.

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DESCRIPTION OF PLATES

PLATE 27

FIG. 1. Case 6. The wall of an artery is heavily infiltrated in one area by exudate which extends into the adventitia and intima. The media is disorganized in the involved area. The infiltrating leukocytes vary in type but polymorphonuclear neutrophils predominate. Hematoxylin and eosin stain. × 436.

FIG. 2. Case 1. The wall of a vessel is eccentrically thickened by cellular infiltration which is most abundant in the adventitia but encroaches on the media. The lumen of these vessels is narrowed. There is considerable cellular proliferation and many newly formed collagen fibers. Large mononuclear cells predominate in the exudate. Hematoxylin and eosin stain. × 192.

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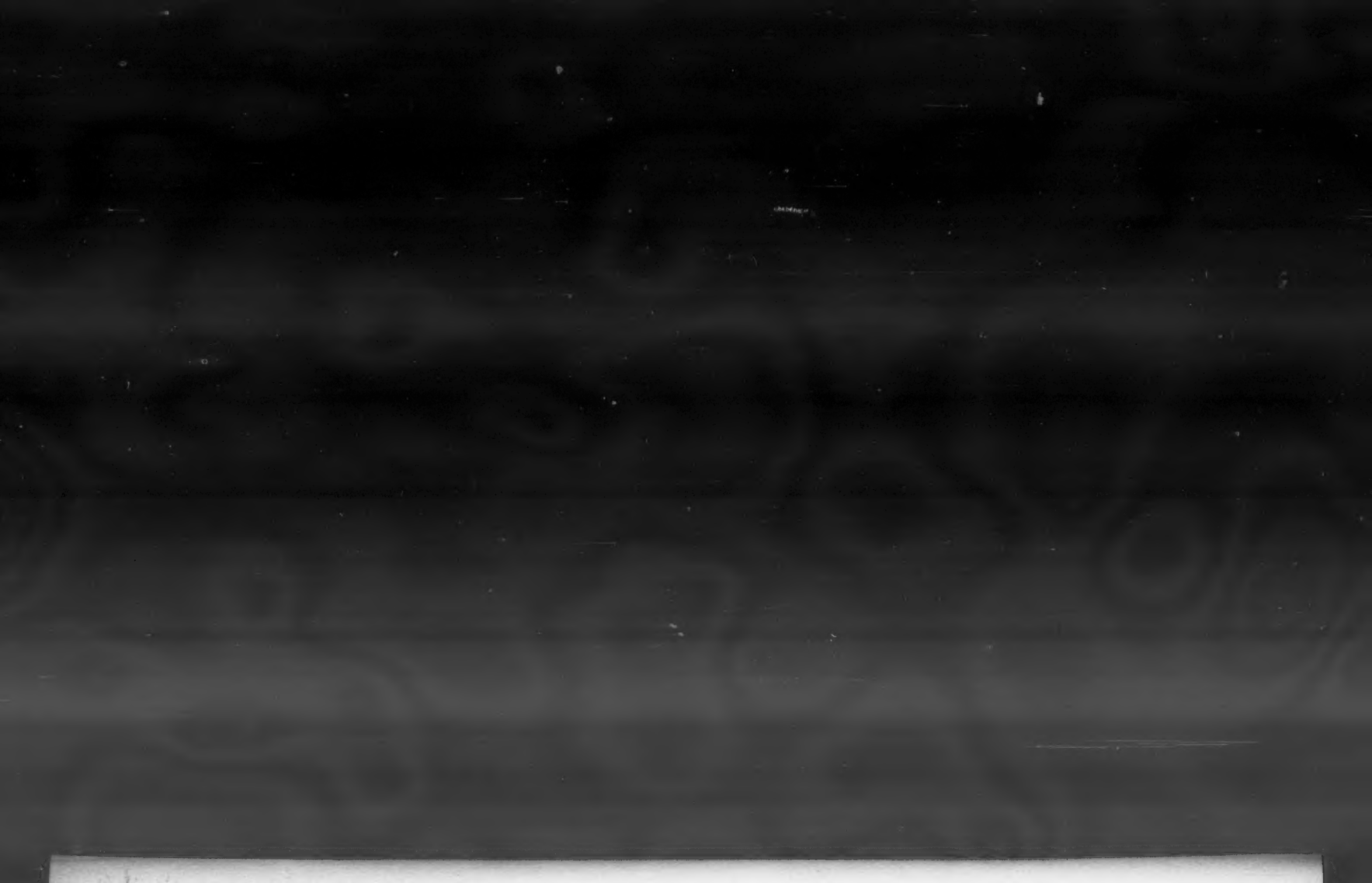

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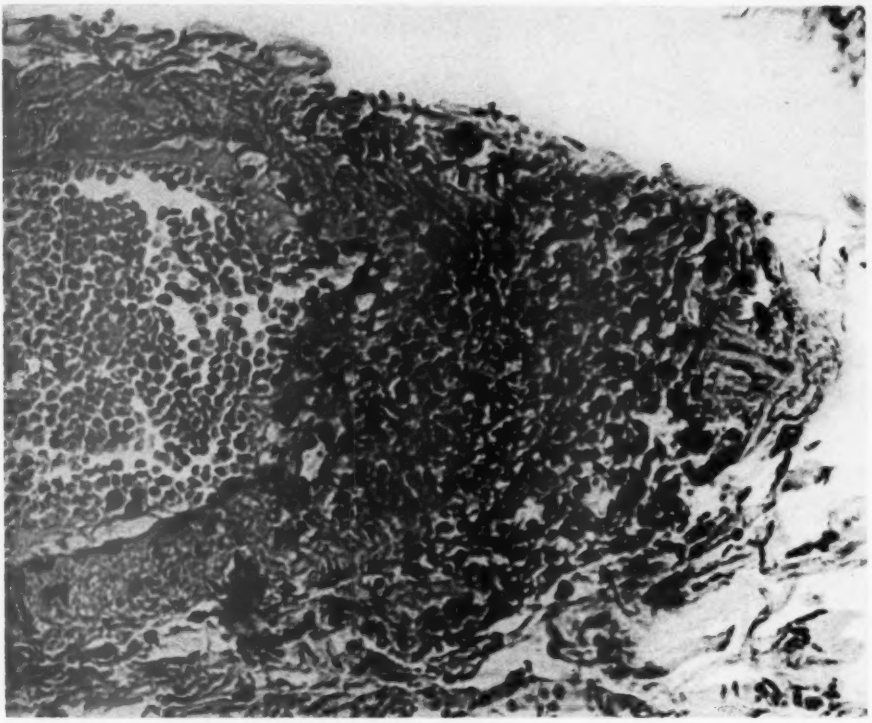

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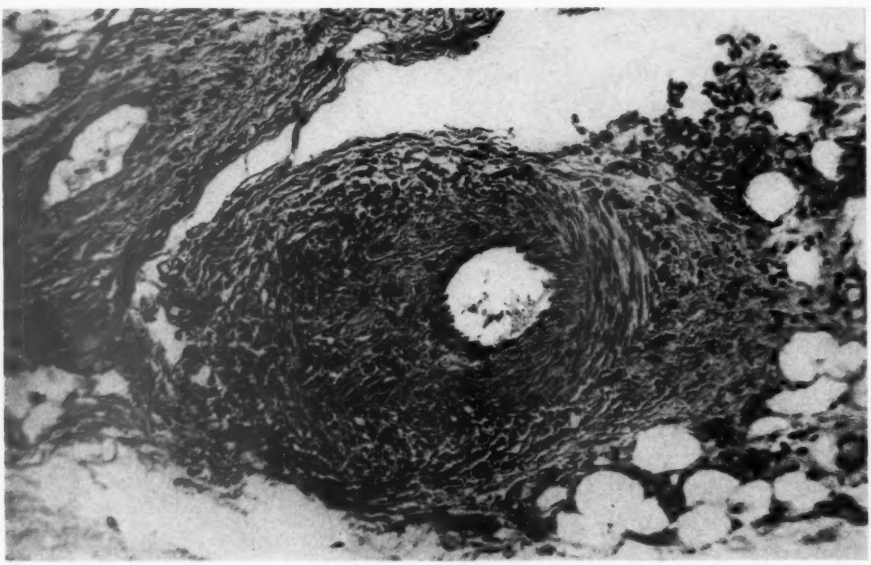

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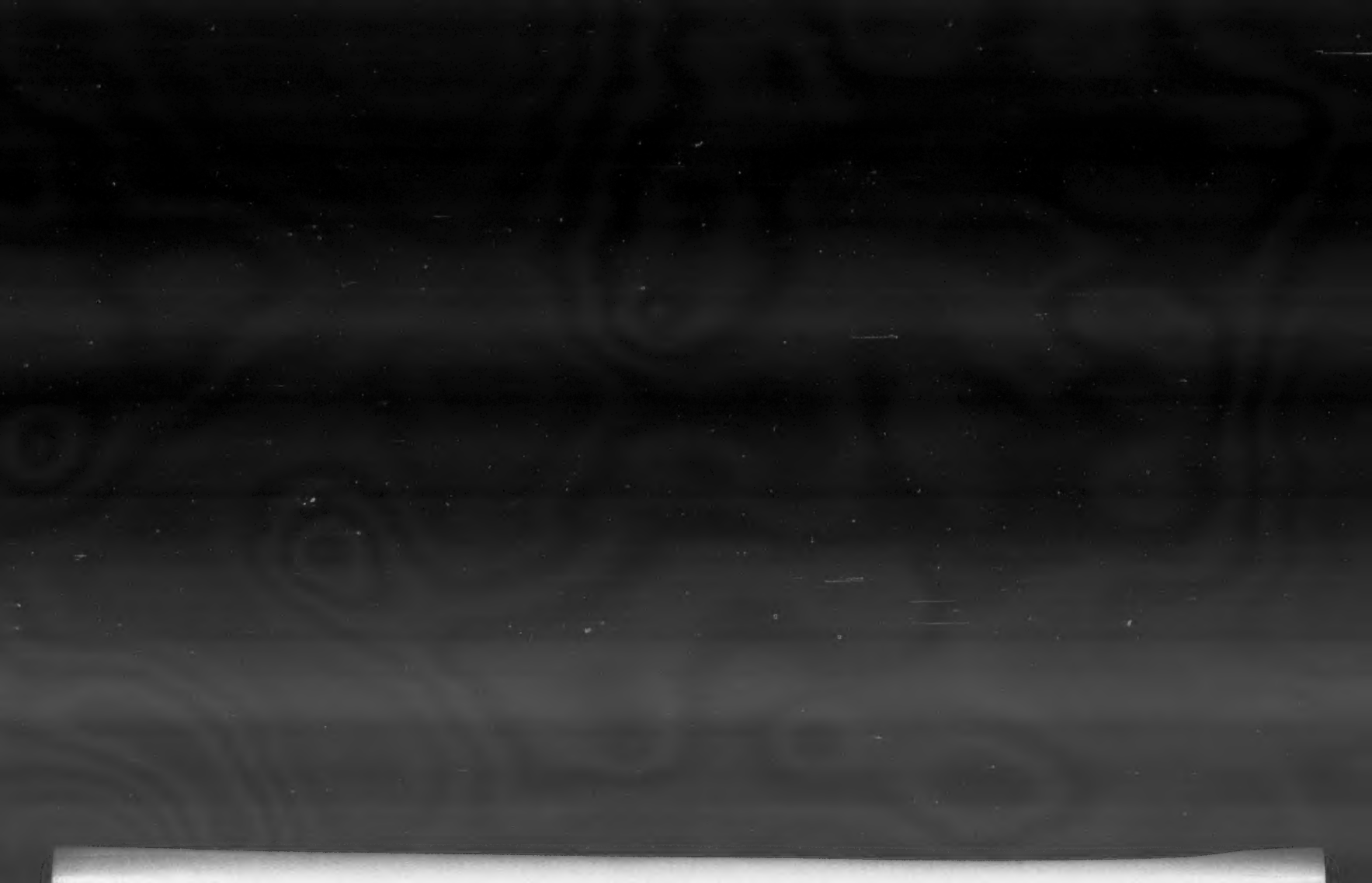
PLATE 28

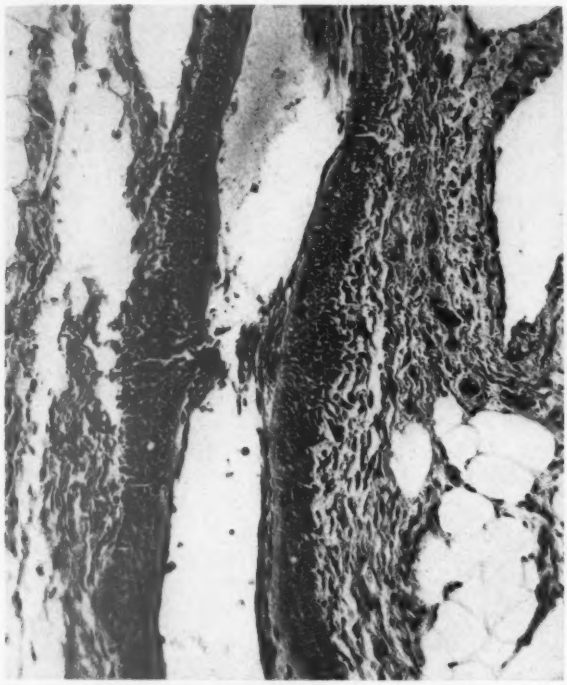
FIG. 3. Case 3. A small segment of an artery cut in longitudinal section is acutely inflamed. Infiltrating leukocytes extend through the media and the latter is disrupted. The endothelium is lifted and stratified. The lumen is still patent. Hematoxylin and eosin stain. × 192.

FIG. 4. Case 5. Acute inflammatory reaction in an arteriole. There is a dense cellular infiltration that extends throughout most of the vessel wall. The lumen is almost obliterated. Hematoxylin and eosin stain. × 210.

FIG. 5. Case 3. Subacute inflammatory reaction in the adventitia of a small artery. The exudate is undergoing organization and replacement by fibrous tissue. The reaction is limited to one margin of the vessel. Hematoxylin and eosin stain. × 210.

FIG. 6. Case 2. An inflammatory reaction extends throughout the wall of an arteriole. The intima is concentrically thickened. The elastica is intact. Hematoxylin and eosin and Weigert's elastic tissue stains. × 210.





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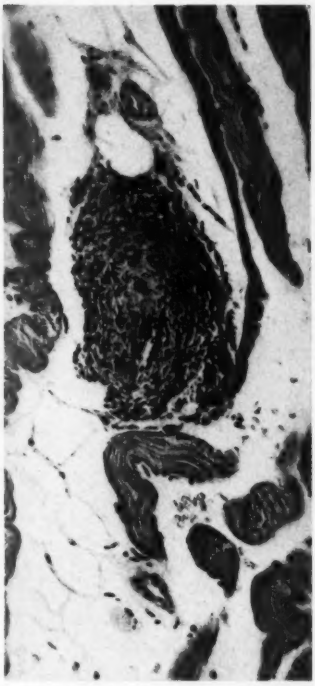

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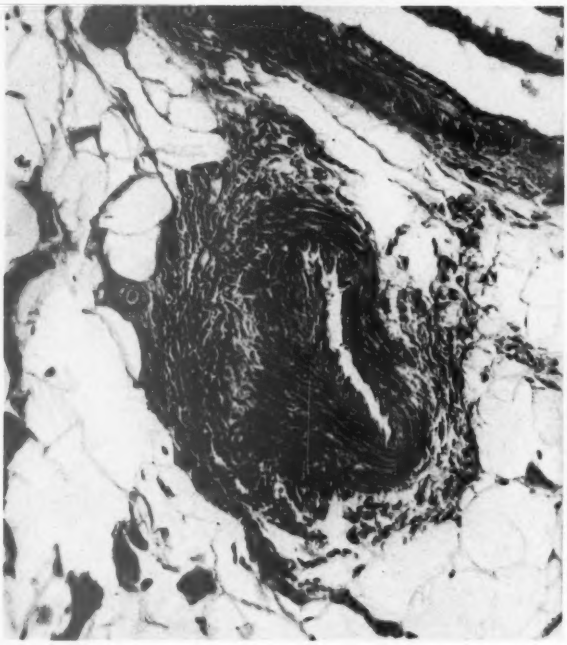

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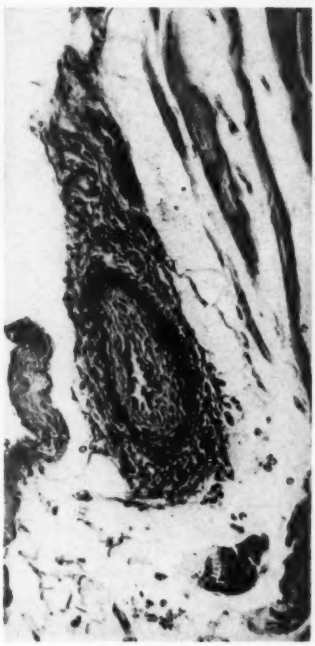

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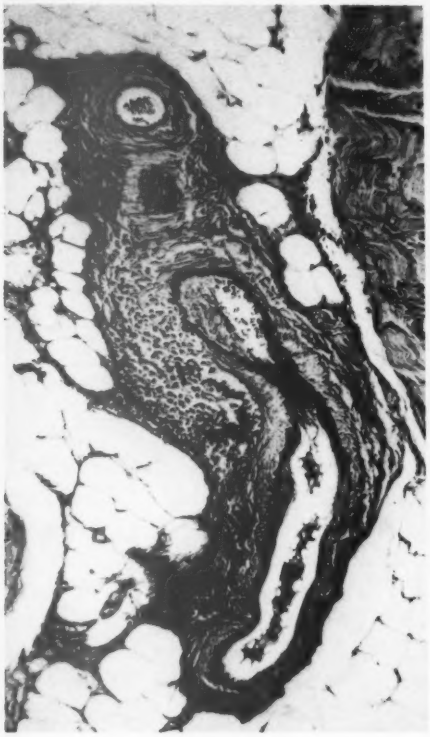
Arteritis in Rheumatoid Arthritis

PLATE 29

FIG. 7. Case 4. The contours of a small artery are distorted by an inflammatory process. The latter is limited to a small segment of the vessel. In the area of cellular infiltration the elastic lamella is thinned out but is not disrupted. Hematoxylin and eosin and Weigert's elastic tissue stains. × 153.

FIG. 8. Case 4. A healed lesion in a small artery. The internal elastic lamella is excessively redundant and tortuous. It is broken at one point and embedded in dense collagen throughout most of its course. Weigert's elastic tissue and van Gieson's connective tissue stains. × 153.

FIG. 9. Acute arteritis in muscle taken for biopsy from a patient with rheumatic heart disease. The media of the vessel has undergone hemorrhagic necrosis. The adventitia is infiltrated by polymorphonuclear leukocytes and large mononuclear cells. Hematoxylin and eosin stain. × 124.



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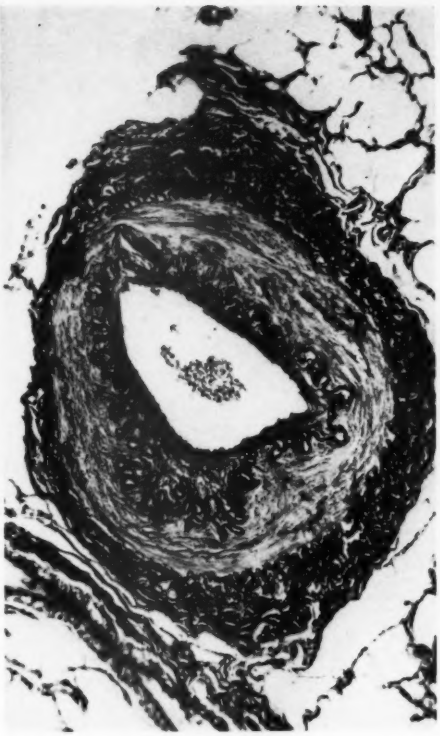

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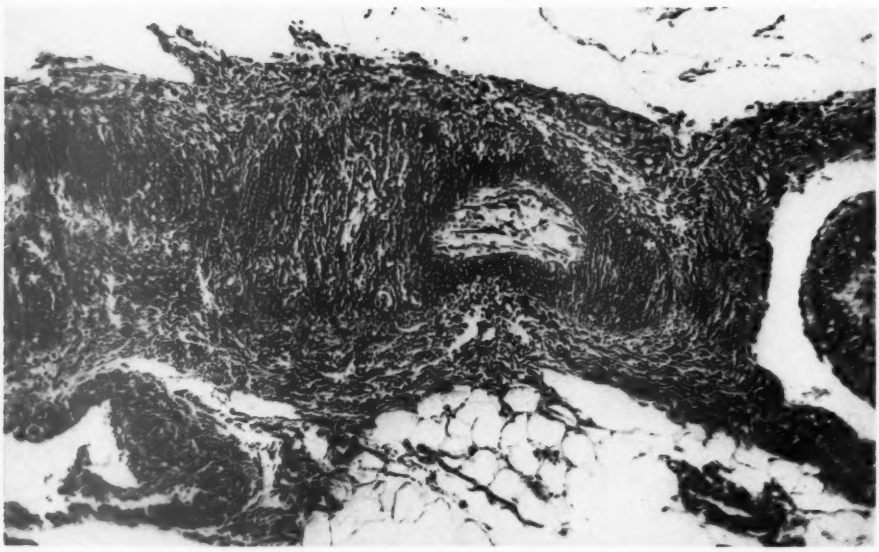

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Sokoloff, Wilens, and Bunim Arteritis in Rheumatoid Arthritis